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BARBARA HARASZTOS

Manuscripts and editorial correspondence should be addressed to

Acta Agronomica Hungarica
Agricultural Research Institute of the
Hungarian Academy of Sciences
H-2462 Martonvásár, Hungary
Phone: (36-22) 569-521
Fax: (36-22) 460-213
E-mail: actaagr@mail.mgki.hu

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ

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HEAVY METALS, SODIUM AND SULPHUR IN ROADSIDE TOPSOILS AND IN THE INDICATOR PLANT CHICORY (*CICHORIUM INTYBUS* L.)

L. SIMON

DEPARTMENT OF LAND AND ENVIRONMENTAL MANAGEMENT, TECHNICAL AND AGRICULTURAL FACULTY, COLLEGE OF NYÍREGYHÁZA, HUNGARY

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The heavy metal (cadmium, Cd; chromium, Cr; copper, Cu; nickel, Ni; lead, Pb and zinc, Zn), sodium (Na) and sulphur (S) contamination of roadside topsoils and the accumulation of these elements in chicory (*Cichorium intybus* L.) as indicator plant was studied at different sites in Nyíregyháza (Hungary) between 1994 and 1996. In roadside urban topsoils (collected from a depth of 0–10 cm at a distance of 0.1 or 0.2–4.0 m from busy roads) the Pb (9–607 mg kg^{-1}), Zn (34–246 mg kg^{-1}) and Cd (0.28–1.12 mg kg^{-1}) concentrations were significantly higher than in similar uncontaminated soils (5.6–9.2 mg kg^{-1} Pb, 33–48 mg kg^{-1} Zn and <0.05–0.30 mg kg^{-1} Cd) located in meadows or gardens far from traffic. The Cr (9.8–26.6 mg kg^{-1}), Ni (8.5–19.4 mg kg^{-1}) and Cu (9.9–32.2 mg kg^{-1}) concentration ranges were similar in roadside topsoils and in uncontaminated soils. In roadside topsoils 4 times more Na and 3 times more S was found than in uncontaminated soils; the Na concentration reached 953 mg kg^{-1} and the S concentration 610 mg kg^{-1}. Wild chicory collected during passive monitoring from the soil sampling sites, accumulated higher levels of Cd, Cr, Ni, Pb and Na in their rhizomes and roots or leaves alongside the roads compared to the uncontaminated sites. During active monitoring, cultivated chicory plants were exposed to the effects of traffic to study the atmospheric deposition of heavy metals. Standardised chicory cultures were placed in plastic pots in the centre of the town or close to highway E 573. After 30 or 60 days of exposure these plants had slightly higher Pb, Cd and Cu concentrations in their leaves than plants placed on an uncontaminated (control) recreational area without traffic. With increasing distance from highway E 573 gradually decreasing levels of Pb were found in the leaves of the indicator plant.

Key words: heavy metals, sodium, sulphur, roadside topsoil contamination, chicory indicator plant

Introduction

Although Hungary is only a moderately industrialised country there is considerable pollution in the most industrialised regions. Polluted areas cover only one-tenth of the country, but affect 40% of the total population (Molnár et al., 1995). During the last 10 years the environmental pollution caused by industry has decreased, but the polluting effects of traffic have increased. Similarly to other countries, Hungarian soils may be contaminated with heavy metals through industrial production, the combustion of fossil fuels, mining, smelting and traffic. The utilisation of sewage sludges, waste waters, agrochemicals and animal manures may also contribute to the heavy metal contamination of arable lands (Kádár, 1995; Molnár et al., 1995).

The heavy metal pollution of urban soils is a global problem threatening the health of the urban population (Thornton, 1991). Besides industrial activities the effects of traffic (combustion of leaded fuel, spill of engine oil, abrasion of tyres, brakes, clutches and automobile parts, evaporation of fuel, etc.) are the main sources of heavy metal contamination on urban and roadside soils (Kádár, 1995). Hungarian roadside soils are contaminated predominantly with lead, but elevated levels of zinc, cadmium, copper, nickel, chromium and other metals were also reported (Kovács and Nyári, 1984; Árkosi and Buna, 1990; Kádár and Koncz, 1993; Dániel et al., 1997). Similar trends were found in other countries (Thornton, 1991; Tiller, 1992; Piron-Frenet et al., 1994; Yläranta, 1995; Garzia et al., 1996). The salting of icy roads is the main source of sodium contamination on roadside soils. The combustion of fuels, mineral oils, coals, etc. contaminates the soils with sulphur (Thornton, 1991; Kádár, 1995).

For the passive or active monitoring of the roadside heavy metal contamination different kinds of plants have been used including lichens (Tuba and Csintalan, 1993), mosses (Markert et al., 1996), weeds (Djingova and Kuleff, 1993; Ötztürk and Türkan, 1993; Dániel et al., 1997), grasses (Kádár and Koncz, 1993; Yläranta,1995; Dietl et al., 1996; Garzia et al., 1996), agricultural plants (Nasralla and Ali, 1985; Yläranta, 1995; Köles et al., 1997), trees and shrubs (Kovács et al., 1981; Ötztürk and Türkan, 1993).

Chicory (*Cichorium intybus* L.) is a perennial weed belonging to the *Compositae* family (Fig. 1). This weed, which grows to a height of 15–200 cm, is frequently found at the edges of roads, in uncultivated areas, on arable land, in gardens, meadows, pastures and embankments. Chicory is native and widespread in the temperate zones of Europe, Near Asia and North Africa. Nowadays chicory is widespread all over the world, and has become a cosmopolitan weed. Chicory flowers from July to October. Its typical bright blue flowers are easy to recognise, which could be helpful in monitoring studies. Cultivated forms are salad chicory (*Cichorium intybus* L. var. *foliosum*), grown as a salad vegetable, and root chicory (*Cichorium intybus* L. var. *radicosum*), utilised as an industrial raw material (coffee substitute, insulin production) (Ujvárosi, 1973). Chicory has been used as indicator plant for the cadmium contamination of soils or nutrient solution (Martin et al., 1996; Simon et al., 1996). It also indicated the excess of Cd, Mn and Zn in municipal sewage sludge compost used as a soil amendment (Simon et al., 1997) and accumulated considerable amounts of Cd, Cu, Cr and Zn from soil contaminated with galvanic mud (Simon, 2001). Chicory collected on roadside areas had the highest Cu concentrations in the leaves among the tested indicator plants (Dániel et al., 1997). In a similar study chicory leaves accumulated elevated levels of Pb, Zn and Cd along the highways (Ötztürk and Türkan, 1993). Since wild and cultivated forms of chicory can be found widely and have the ability to tolerate a broad range of soil and climatic conditions, this plant has the potential to be an international standard indicator species for heavy metal contamination.

Considering the above facts the aims of the present work were the following:

- a survey of the heavy metal (Cd, Cu, Cr, Ni, Pb and Zn), sodium and sulphur contamination of urban topsoils alongside the busy roads of Nyíregyháza, Hungary,
- the assessment of how these elements enter the biosphere, with the help of chicory (*Cichorium intybus* L.) as indicator plant.

Materials, methods and study area

Passive monitoring with wild chicory

Passive monitoring was carried out during August 1995 and August 1996 by collecting wild chicory (*Cichorium intybus* L.) plant samples (Fig. 1) to study the heavy metal contamination of roadside urban soils in Nyíregyháza. Nyíregyháza has 120,000 inhabitants and is located in the northeastern part of Hungary at longitude 21°41' E and latitude 47°58' N, 100–120 m above the Baltic sea level. The city is moderately industrialised without factories polluting the environment directly with heavy metals. Soil samples, and where available chicory samples, were collected from uncontaminated control sites far from traffic (sites 1–3), or at sites exposed to the effects of traffic (sites 4–15) (Fig. 2). Site 1 was in a meadow 150 m from highway E 573, site 2 was in an allotment garden 20 m from the nearest road, and site 3 was in the demonstration garden of the College of Agriculture in Nyíregyháza, 25 m from the nearest road. Sites 4–15 were at different locations of Nyíregyháza (Fig. 2) close to the roads, preferably in places where flowering wild chicory plants were to be found. Soil samples were also collected very close (at a distance of 0.1 m) to the edge of the roads at sites 4–15. At each site three parallel soil samples (each made by combining 10 subsamples) were collected from the 0 to 10 cm layer. Several basic characteristics of the soils, namely clay+silt (<0.02 mm) content, pH_{KCl} and organic carbon % were determined according to Hungarian standards.

Fig. 1. Wild form of chicory (*Cichorium intybus* L.) used for passive monitoring (source Ujvárosi, 1973)

Active monitoring with cultivated chicory

Active monitoring was carried out between June 1 and August 2, 1994 using a cultivated form of chicory (*Cichorium intybus* L. var. *foliosum* Hegi, cv. Wild) to study the atmospheric heavy metal deposition in the town of Nyíregyháza.

Thirty-day-old chicory seedlings were planted in plastic pots (13 cm diameter and 16 cm height) containing 1.3 kg of a loamy sandy slightly acidic brown forest soil (collected in the demonstration garden of the College of Agriculture in Nyíregyháza) uncontaminated with heavy metals. The soil characteristics were the following: clay+silt (<0.02 mm) content 15.8 %, pH_{KCl} 6.6, organic carbon 0.75 %, CEC 18.1 meq 100 g^{-1}, P 0.9 g kg^{-1}, K 4.0 g kg^{-1}, Ca 31.3 g kg^{-1}, Mg 8.0 g kg^{-1}, Fe 18.4 g kg^{-1}, Cd 0.3 mg kg^{-1}, Cr 18.7 mg kg^{-1}, Cu 15.3 mg kg^{-1}, Mn 503 mg kg^{-1}, Ni 10.2 mg kg^{-1}, Pb 11.0 mg kg^{-1} and Zn 52.7 mg kg^{-1} (all elements were extracted with cc. HNO_3 and H_2O_2). The plants in the plastic pots were grown for the next 30 days in a greenhouse. When the plants were 60 days old and had 7–8 true leaves, pots with 3 uniformly sized plants were selected, and were placed at the monitoring sites (Fig. 3):

Site 1: Recreational area (at a height of 0 m),
Site 2: Recreational area (at a height of 6 m),
Site 3: Centre of the town (at a height of 0 m),
Site 4: Centre of the town (at a height of 6 m),
Site 5: Highway E 573 (at a height of 0 m).

At all sites 10 pots with a total of 30 plants were set out. The pots at sites 1 and 2 were placed in the Császárszállás recreational area, located 6 km south of Nyíregyháza (Fig. 3), which was considered as a control uncontaminated area since it is far away from busy main roads. The plants at sites 3 and 4 were placed in the centre of the town close to very busy roads (Fig. 3). The pots were placed on the soil surface (sites 1 and 3) or at a height of approximately 6 m (sites 2 and 4) in order to study the vertical movement and deposition of airborne heavy metal contaminants. Half of the plants were collected after 30 days of exposure, and the remaining half was collected after 60 days. The plants at site 5 were placed on the soil surface at distances of 2.5, 8.0 and 16.5 m from the west side of highway E 573 (Fig. 3). The plants here were exposed to the effects of traffic for 30 days before elemental analysis. Highway E 573 runs from north to south, and the average traffic density on this section of the road is 7400 vehicles per day (data from the Public Road Maintenance Company in Nyíregyháza). There were no natural obstacles (e.g. bushes) in the monitoring area to prevent heavy metal deposition on the plants. The prevailing wind direction was north–northeast in the studied area.

All plants were watered regularly with tap water during the monitoring period.

Elemental analysis of soil and plant samples

The collected plant samples were taken to the laboratory, and were divided into rhizomes and roots, and leaves. The rhizomes and roots of chicory were thoroughly washed with running tap water and rinsed 3 times in distilled water. The leaves were not washed, but solid pollutants were carefully removed from the leaf surface with a brush. The plant and soil samples were dried, milled, sieved (<0.5 mm), and digested with cc. HNO_3 and H_2O_2 (3:1 v/v) before elemental analysis (Simon et al., 1997). The elemental composition of the soil and plant samples was determined by inductively coupled argon plasma emission spectrometry (ICAP, model Labtam 8440M, Australia) in triplicate. The lead content of the plant samples was determined by graphite furnace atomic absorption spectrometry (GF-AAS, model Unicam 939 QZ, Cambridge, UK) in triplicate. For the validation of the plant analysis the certified reference material CRM 281 rye grass (Commission of the European Community, Community Bureau of Reference, Brussels) was used. The elemental analysis was done in the Central Chemical Laboratory, Debrecen University of Agricultural Sciences, Debrecen, Hungary.

Statistics

Data processing and statistical analysis of the experimental data using Student's t-test was done using MS Excel 5.0 software (Microsoft Excel 5.0 9, 1993).

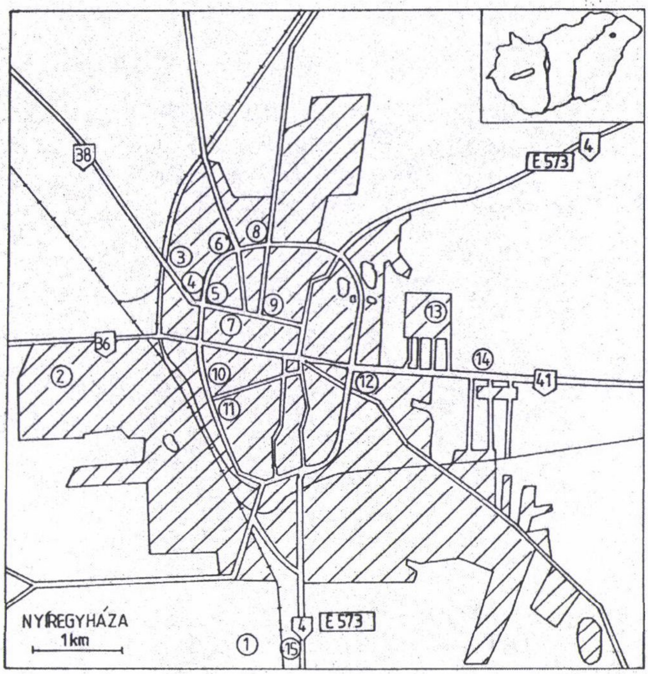

Fig. 2. Map of Nyíregyháza with sites (1–15) for passive monitoring of heavy metal contamination

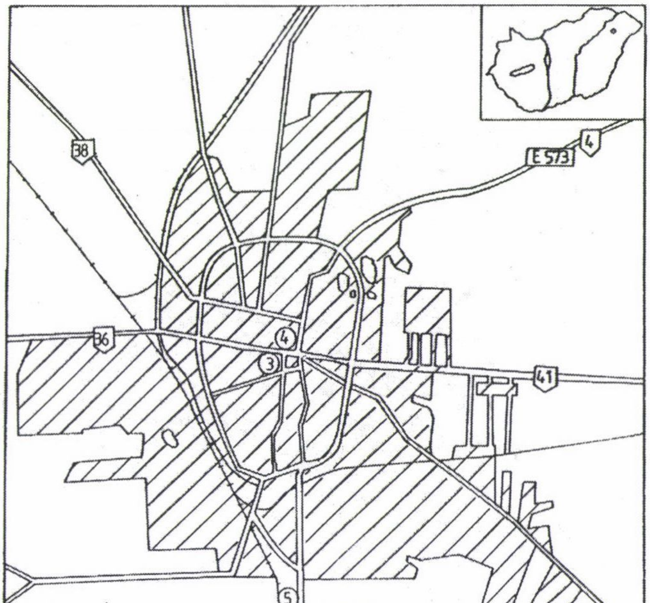

Fig. 3. Map of Nyíregyháza with sites (1–5) for active monitoring of heavy metal contamination

Results and discussion

Heavy metals, sodium and sulphur in roadside topsoils and in wild chicory

The pH of the uncontaminated soils at sites 1–3 ranged from pH_{KCl} 6.6 to pH_{KCl} 7.5, the clay+silt (<0.02 mm) content varied between 10.0% and 16.0%, and the organic carbon content ranged from 0.46% to 0.98%. At sites 4–15 the roadside topsoils had the following characteristics: pH_{KCl} 7.0–7.8, clay+silt (<0.02 mm) content 8.0%–12.8 %, organic carbon content 0.93%–1.45%.

The heavy metal, sodium and sulphur concentrations in urban topsoils in Nyíregyháza are shown in Table 1 for the sites of monitoring. Compared to uncontaminated sites (1–3), urban topsoils (sites 4–15) close to busy roads had significantly higher Pb, Zn and Cd levels. The contamination was higher at 0.1 m from the roads than at 0.2–4 m. The Pb contamination was the most serious, with a median concentration of 129 mg kg^{-1} in the investigated urban soils, and minimum and maximum values of 9 mg kg^{-1} and 607 mg kg^{-1}. The lowest value was found at site 13, which is located in a residential area with blocks of flats. Here, the topsoil was replaced after the construction of the buildings was completed in 1989. The time elapsed was probably not long enough for significant lead contamination of the topsoil. The highest Pb concentration was found at the edge of a safety island at site 5, where the average traffic density was 20,850 vehicles per day (data from the Public Road Maintenance Company in Nyíregyháza). At other sites exposed to the effects of traffic the lead contamination of the soil was also significant; the range of lead contamination values was similar to those found by other Hungarian (Kovács and Nyári, 1984; Árkosi and Buna, 1990; Kádár and Koncz, 1993; Dániel et al., 1997) and foreign (Tiller, 1992; Piron-Frenet et al., 1994; Garzia et al., 1996) investigators in the topsoils alongside busy roads. Presumably the traffic contributed to the greatest extent to the Pb contamination of roadside topsoils in Nyíregyháza, since leaded fuel was used exclusively in Hungary for decades.

Elevated levels of zinc and cadmium were also detected in the topsoils at sites 4–15 compared to the uncontaminated soils (sites 1–3) (Table 1). This surplus of Zn and Cd may originate from the abrasion of tyres and the galvanised parts of vehicles (Kádár, 1995). The concentrations of chromium, copper and nickel were similar in uncontaminated soils and roadside topsoils (Table 1). Besides the direct effects of traffic, atmospheric wet deposition may also contribute to the heavy metal contamination of urban soils (Alloway, 1990). At the Meteorological Observation Station in Napkor, 15 km from Nyíregyháza, 0.57 mg Cd m^{-2}, 3.7 mg Cu m^{-2}, 0.72 mg Ni m^{-2}, 7.2 mg Pb m^{-2} and 22 mg Zn m^{-2} annual wet deposition was measured in 1993 (Mészáros et al., 1993). In Hungary the average wet deposition and air concentration of lead and cadmium has gradually decreased in recent years; in 1994 these values were 4 mg m^{-2} $year^{-1}$ and 30 ng m^{-3} for lead and 0.1 mg m^{-2} $year^{-1}$ and 0.4 ng m^{-3} for cadmium (Bozó and Boronka, 1996).

Table 1

Heavy metal, sodium and sulphur concentrations (mg kg^{-1}) in the urban topsoils at various monitoring sites (Nyíregyháza, Hungary, 1995, 1996)

Location of monitoring	Distance from the road (m)	Cd	Cr	Cu	Ni	Pb	Zn	Na	S
Site 1	150	<0.05	7.8	53.5	9.3	5.6	33	83	< 2
Site 2	25	0.20	19.0	20.1	18.6	5.7	48	89	146
Site 3	20	0.30	12.2	15.0	14.9	9.2	41	443	190
Site 1–3	Median	0.20	12.2	20.1	14.9	5.7	41	89	146
Site 4	0.1	0.65	26.6	26.5	12.1	361	145	476	504
Site 4	1.6–2.9	0.57	18.5	28.4	12.2	237	179	827	499
Site 5	0.1	0.79	19.8	25.7	11.4	607	116	390	427
Site 5	1.2-1.5	0.46	18.0	24.5	15.0	280	84	683	610
Site 6	0.1	0.70	17.3	32.2	11.0	277	134	253	309
Site 6	0.2-1	0.33	15.1	19.0	13.7	34	72	422	577
Site 7	0.1	0.99	14.2	20.1	10.7	237	142	384	401
Site 7	2.0	1.11	14.2	19.6	12.5	70	177	391	306
Site 8	0.1	0.53	16.8	24.3	9.6	327	100	372	502
Site 8	0.5–0.7	0.88	14.0	23.8	11.9	191	118	540	448
Site 9	0.1	0.58	14.8	28.8	10.6	446	105	433	301
Site 9	0.3–1.8	0.50	18.3	17.3	12.7	73	246	790	530
Site 10	0.1	0.55	14.7	18.9	10.3	131	109	291	495
Site 10	1.6-2.4	1.12	18.1	16.3	14.8	129	117	693	548
Site 11	0.1	0.67	15.7	18.4	12.8	116	103	407	391
Site 11	2.0	0.29	15.6	17.7	13.6	30	68	953	590
Site 12	0.1	0.28	12.4	12.6	15.7	52	68	678	239
Site 12	4.0	0.37	13.6	18.6	12.8	103	75	233	385
Site 13	1.5	0.38	16.1	9.9	19.7	9	34	159	59
Site 14	1.5–3.0	0.51	16.7	22.3	17.9	52	92	273	305
Site 15	2.0	0.50	9.8	12.3	8.5	94	55	262	253
Site 4–15	Median	0.55	15.7	19.6	12.5	129	105	407	427
Site 4–15	Minimum	0.28	9.8	9.9	8.5	9	34	159	59
Site 4–15	Maximum	1.12	26.6	32.2	19.4	607	246	953	610

Each value represents the mean of 3 replications. Element concentrations were determined from cc. HNO_3 and H_2O_2 extracts and were calculated on a dry matter basis.

In Hungary, the regulatory limits for heavy metal concentrations in arable soils after sewage sludge deposition are 1–3 mg kg^{-1} for Cd, 75–100 mg kg^{-1} for Cr, 75–100 mg kg^{-1} for Cu, 50 mg kg^{-1} for Ni, 100 mg kg^{-1} for Pb and 200–300 mg kg^{-1} for Zn, depending on the soil properties (Kádár, 1995). The median Pb concentration (129 mg kg^{-1}) of the roadside topsoils in Nyíregyháza exceeds the Hungarian regulatory limit for arable soils (regulatory limits for urban soils have not yet been established in Hungary) and is higher than the domestic and international average Pb concentrations in soils (Alloway, 1990; Kádár, 1995).

The median concentration of sodium was 89 mg kg^{-1} in uncontaminated soils and more than 4 times higher (407 mg kg^{-1}) along the busy roads (Table 1). A similar trend was observed in sulphur concentrations (Table 1), with 3 times more S in roadside topsoils (median 427 mg kg^{-1}) than in soils located far from traffic (median 146 mg kg^{-1} S). The surplus of Na and S in the soils could definitely be attributed to human activity (i.e. road salting, fuel combustion).

Table 2
Heavy metal, sodium and sulphur accumulation ($\mu g\ g^{-1}$) in wild chicory (*Cichorium intybus* L.) collected at various monitoring sites (Nyíregyháza, Hungary, 1995, 1996)

Location of monitoring	Distance from the road (m)	Cd	Cr	Cu	Ni	Pb*	Zn	Na	S
		Rhizomes and roots							
Site 1	150	<0.05	<0.20	18.9	<0.25	0.10	34	763	1813
Site 2	25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Site 3	20	<0.05	<0.25	11.9	<0.1	1.44	19.4	164	873
Site 4	1.6–2.9	0.28	2.14	12.9	0.90	0.33	49	9400	1467
Site 5	1.2–1.5	0.24	1.15	6.6	0.99	0.97	100	7033	1940
Site 6	0.2–1.0	0.31	1.31	23.2	1.87	0.46	54	8833	2403
Site 7	2.0	<0.05	<0.20	5.8	<0.25	0.75	21	3173	1180
Site 8	0.5–0.7	0.83	2.68	11.3	1.47	1.49	54	20833	2257
Site 9	0.3–1.8	0.12	2.95	13.0	1.42	0.35	73	9967	1677
Site 10	1.6–2.4	0.30	2.02	2.9	1.34	0.66	46	11133	1397
Site 12	4.0	<0.05	0.49	7.7	<0.25	4.03	24	5395	1680
Site 13	1.5	<0.05	<0.20	21.2	<0.25	0.81	33	5500	1345
Site 14	1.5–3.0	<0.05	<0.20	3.0	<0.25	6.16	8	4388	923
Site 15	2.0	0.18	1.06	18.5	<0.25	6.52	37	3363	1582
Site 4–15	Median	0.18	1.15	11.3	0.9	0.81	46	7033	1582
Site 4–15	Minimum	<0.05	<0.20	2.9	<0.25	0.33	8	3173	923
Site 4–15	Maximum	0.83	2.95	23.2	1.87	6.52	100	20833	2403
		Leaves							
Site 1	150	<0.05	<0.20	12.5	<0.25	0.20	87	145	8333
Site 2	25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Site 3	20	<0.05	<0.15	12	<0.1	3.4	53	139	4747
Site 4	1.6–2.9	0.70	2.89	13.2	1.85	1.48	162	2700	5500
Site 5	1.2–1.5	0.49	2.42	12.8	1.50	4.21	72	3603	5767
Site 6	0.2–1.0	0.27	2.71	60.0	1.74	3.41	84	1537	4407
Site 7	2.0	<0.05	0.58	11.1	<0.25	0.81	81	539	6628
Site 8	0.5–0.7	0.75	2.01	58.0	2.20	1.60	69	4527	8367
Site 9	0.3–1.8	0.36	1.64	32.1	1.45	1.60	91	1580	4760
Site 10	1.6–2.4	0.74	1.76	24.8	1.12	2.45	64	5300	4510
Site 12	4.0	<0.05	<0.20	2.5	<0.25	4.98	35	1253	4485
Site 13	1.5	<0.05	<0.20	29.2	<0.25	2.83	47	3677	4993
Site 14	1.5–3.0	0.09	0.25	14.2	<0.25	1.60	63	4647	5991
Site 15	2.0	1.21	2.7	17.2	0.31	1.63	95	6582	3893
Site 4–15	Median	0.36	1.76	17.2	1.12	1.63	72	3603	4993
Site 4–15	Minimum	<0.05	<0.20	2.5	<0.25	0.81	35	539	3893
Site 4–15	Maximum	1.21	2.89	60.0	2.2	4.98	162	6582	8367

n.d. = no data, no chicory was found. Each value represents the mean of 3 replications. Element concentrations were determined from cc. HNO_3 and H_2O_2 extracts and were calculated on a dry matter basis. Pb*: determined by graphite furnace atomic absorption spectrometry (GF-AAS).

Wild chicory collected from the soil sampling sites indicated the pollution of the environment with heavy metals (Table 2). The plants were exposed to both the heavy metal contamination of roadside soils and atmospheric heavy metal deposition. The median concentrations of Cd, Cr, Ni and Pb in chicory rhizomes and roots or leaves were higher in the neighbourhood of roads than at uncontaminated sites 1 or 3 (at site 2 no chicory was found). These results confirm those of Ötztürk and Türkan (1993) who found elevated levels of Pb, Zn and Cd in the leaves of chicory collected along busy highways. The accumulation of these heavy metals (Cd, Cr, Ni and Pb) was also reported in other indicator plant species collected alongside roads with heavy vehicular traffic (Nashralla and Ali, 1985; Djingova and Kuleff, 1993; Kádár and Koncz, 1993; Tuba and Csintalan, 1993; Yläranta, 1995; Dietl et al., 1996; Dániel et al., 1997; Köles et al., 1997).

Wild chicory rhizomes and roots or leaves also indicated the excess of Na in the soil (Table 2), while the increase in S concentrations in chicory organs was ambiguous.

Heavy metals in cultivated chicory

Tables 3 and 4 show the heavy metal accumulation rate in cultivated chicory plants placed for 30 or 60 days in the inner town of Nyíregyháza or in a recreational (uncontaminated) area. Statistically higher levels of Cd and Pb were found in the leaves of chicory exposed to the effects of traffic for 30 days in the centre of the town than in the control area. Changes in Cr, Cu or Zn concentrations could be attributed to the mineral nutrition anomalies of chicory. After 60 days of exposure the plants in the inner town had higher Pb and Cu levels in their leaves. Lead concentration also increased at a height of 6 m (Table 4).

Table 3

Heavy metal accumulation ($\mu g\ g^{-1}$) in cultivated chicory (*Cichorium intybus* L. var. *foliosum* Hegi) after 30 days of exposure at various monitoring sites in Nyíregyháza (Hungary)

Location of monitoring	Height from the ground	Cd	Cr	Cu	Ni	Pb*	Zn
		Rhizomes and roots					
Recreational area (Site 1)	0 m	1.05*	1.8*	8.1	3.4	0.61	14.3
Centre of the town (Site 3)	0 m	0.95	1.6	9.1**	3.7	0.89*	23.3
Recreational area (Site 2)	6 m	0.93	1.6	9.9	3.4	0.80	18.4
Centre of the town (Site 4)	6 m	0.95	2.0*	17.4**	4.0*	0.82	34.5**
		Leaves					
Recreational area (Site 1)	0 m	0.98	1.8	8.4	3.4	0.60	19.5
Centre of the town (Site 3)	0 m	1.06*	1.9	10.9**	3.7	1.42*	29.4*
Recreational area (Site 2)	6 m	1.13	1.7	10.2	3.1	0.72	54.5
Centre of the town (Site 4)	6 m	1.89**	2.8*	21.1**	9.2	1.87**	59.5

Student's t-test. Statistically significant at *: $P<0.05$, **: $P<0.01$ level. Each value represents the mean of 3 replications. Element concentrations were determined from cc. HNO_3 and H_2O_2 extracts and were calculated on a dry matter basis. Pb*: determined by graphite furnace atomic absorption spectrometry (GF-AAS).

Table 4

Heavy metal accumulation (μg g^{-1}) in cultivated chicory (*Cichorium intybus* L. var. *foliosum* Hegi) after 60 days of exposure at various monitoring sites in Nyíregyháza (Hungary)

Location of monitoring	Height from the ground	Cd	Cr	Cu	Ni	Pb*	Zn
		Rhizomes and roots					
Recreational area (Site 1)	0 m	0.95	1.4	8.4	4.1	0.68	23.4
Centre of the town (Site 3)	0 m	0.97	1.7	7.9	3.1	0.70	23.8
Recreational area (Site 2)	6 m	0.94	1.4	11.0	3.6	0.74	17.4
Centre of the town (Site 4)	6 m	0.90	1.6	11.3	3.5	0.93*	16.5
		Leaves					
Recreational area (Site 1)	0 m	1.08	1.5	15.2	5.5	0.59	37.3
Centre of the town (Site 3)	0 m	1.01	1.7	12.4	4.2	0.84*	32.5
Recreational area (Site 2)	6 m	1.06	1.4	11.1	3.0	0.57	33.6
Centre of the town (Site 4)	6 m	1.09	1.7	14.4*	3.5	1.17*	47.0

Student's t-test. Statistically significant at *: $P < 0.05$ level. Each value represents the mean of 3 replications. Element concentrations were determined from cc. HNO_3 and H_2O_2 extracts and were calculated on a dry matter basis. Pb*: determined by graphite furnace atomic absorption spectrometry (GF-AAS).

Figure 4 shows the lead concentrations in rhizomes+roots and in the leaves of chicory placed at various distances from highway E573. Plants exposed to the effects of traffic for 30 days had higher Pb levels in their leaves than plants placed on an uncontaminated recreational control area far away from busy roads. With increasing distance from the road, Pb levels gradually decreased in the leaves of the indicator plant (Fig. 4).

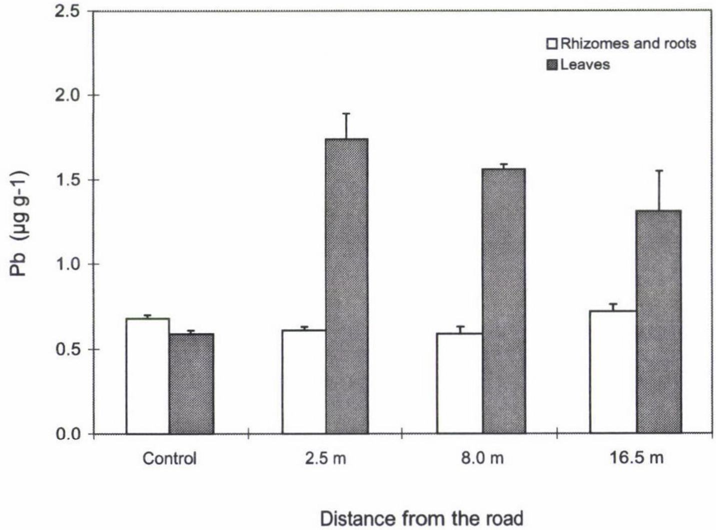

Fig. 4. Lead accumulation in chicory placed at different distances from highway E573 near to Nyíregyháza after 30 days of exposure

The inverse relationship between the lead concentration in vegetation and the distance from the road is well documented (Nashralla and Ali, 1985; Thornton, 1991; Djingova and Kuleff, 1993; Kádár and Koncz, 1993; Ötztürk and Türkan, 1993; Tuba and Csintalan, 1993; Yläranta, 1995; Dániel et al., 1997; Köles et al., 1997), and is confirmed by the present observations. In another study (Markert et al., 1996), however, the expected correlation between the Pb emission of traffic and the Pb concentrations in moss species could not be demonstrated with certainty.

The levels of other heavy metals (Cd, Cr, Cu, Ni and Zn) in chicory remained unchanged (data not shown). Dietl et al. (1996) found moderate lead accumulation in standardised grass cultures placed along roads with heavy traffic, but the bioaccumulation of cadmium was not observed at all. Our results are in agreement with these findings.

The lead concentration of the petrol (gasoline) sold in Hungary was reduced from 0.6 g dm^{-3} to 0.4 g dm^{-3} during the 1980s, and since 1992 this value has been only 0.15 g dm^{-3}. The total estimated lead emission of the traffic gradually decreased from 700 tons in 1980 to 100 tons in 1994. In 1997 only 29 % of the total petrol sold was leaded, and since April 1999 no leaded fuels have been produced in Hungary (data from the Hungarian Ministry of the Environment). In spite of these efforts, in several cities with heavy traffic the lead concentration of the air is still twice as high as the limit value. The Hungarian limit value for the Pb concentration in the flying dust of the air is 0.3 μg Pb dm^{-3} per 24 hours. The National Public Health and Medical Officer's Service (ÁNTSZ) regularly measures the lead concentration attached to flying dust in the air in bigger Hungarian towns. In Nyíregyháza the ÁNTSZ sampling point was very close to sites 3 and 4, where the active monitoring with chicory was done to study the atmospheric heavy metal contamination of the town. During our study period (June–July, 1994) the median value of the Pb concentration in flying dust was 0.12 μg m^{-3}, with a minimum value of 0.02 μg m^{-3} and a maximum of 0.13 μg dm^{-3} (data were based on 6 measurements). The median value of the annual data was consistently around 0.1 μg Pb dm^{-3} every year from 1994 to 1997 (data by permission of the National Public Health and Medical Officer's Service in Szabolcs-Szatmár-Bereg county). On the basis of the above data it can be assumed that the elevated Pb levels in chicory leaves came mainly from the combustion of leaded petrol.

Conclusions

Roadside topsoils exposed to the effects of traffic in Nyíregyháza are contaminated with Pb, Zn, Cd, Na and S, the concentration of which reached 607 mg kg^{-1}, 246 mg kg^{-1}, 1.12 mg kg^{-1}, 6582 mg kg^{-1} and 8367 mg kg^{-1}, respectively. Elevated levels of Cd, Cr, Ni, Pb and Na were found in the organs of wild chicory collected alongside busy roads during passive monitoring. Cultivated chicory, placed in the inner town close to busy roads during an active monitoring, accumulated slightly higher levels of Pb, Cd and Cu in the leaves compared to plants placed in an uncontaminated recreational area. With increasing distance from highway E 573 the Pb concentration gradually decreased in the leaves of cultivated chicory.

Acknowledgements

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INFLUENCE OF WATER STRESS CONDITIONING ON PHOTOSYNTHETIC WATER STRESS RESPONSE OF SWITCHGRASS (*PANICUM VIRGATUM* L.) AND TALL FESCUE (*FESTUCA ARUNDINACEA* SCHREB.)

Z. KISS[1] and D. D. WOLF[2]

[1]DEPARTMENT OF CROP AND SOIL ENVIRONMENTAL SCIENCES, DEBRECEN UNIVERSITY, DEBRECEN, HUNGARY;
[2]VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY, BLACKSBURG, USA

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The objective of this study was to investigate the influence of water stress conditioning on the photosynthesis response of switchgrass (*Panicum virgatum* L.) and tall fescue (*Festuca arundinacea* Schreb.) to moisture deficiency. Tillers of the two species were grown in the same, controlled, environment and were subjected to three conditioning water stress cycles, or were kept well watered. After drought conditioning all plants were subjected to moisture deficiency while photosynthesis and leaf water potential were monitored. Measurements were taken between –0.8 and –4.0 MPa and the rate of water stress was 0.49 MPa/day. The conditioning of switchgrass produced a 26% reduction in the photosynthesis rate during drought, while that of tall fescue produced a 57% reduction in photosynthesis. Both species maintained elongation and photosynthesis down to lower leaf water potentials after drought conditioning than before conditioning. The conditioning water stress cycles decreased the leaf conductance, mesophyll resistance and transpiration of tall fescue plants after rewatering. The leaf water potential of conditioned switchgrass plants was lower upon rewatering after three conditioning water stress cycles than the leaf water potential of non-conditioned plants, while the leaf conductance, mesophyll resistance and transpiration of conditioned and non-conditioned tillers were equal.

These data indicate an improvement in the drought tolerance of tall fescue and switchgrass plants, emphasize the importance of knowing the previous water stress history of the plants in moisture deficiency experiments, and help to choose proper irrigation management for switchgrass and tall fescue.

Keywords: repeated water stress, elongation, photosynthesis, leaf water potential, transpiration, mesophyll resistance, leaf conductance

Introduction

Warm season grasses, such as switchgrass, give their greatest yield during the hot, dry midsummer days when the production of cool season grasses such as tall fescue declines (Nyakas, 1997; 1999). However, the yield of both grass types is limited by low soil water availability. For water stress survival their management must be different during moisture deficiency. The best management practices can be chosen based on the physiological drought response of these forage grasses.

Many researchers have investigated the photosynthesis responses of warm season and cool season grasses to water stress, separately. Sorghum was the subject of the CO_2 exchange study of Shearman et al. (1972), who concluded that leaf resistance was the most important factor in the photosynthesis limitation of sorghum. Jones and Rawson (1979) found that rapid water stress development

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caused a greater decrease in photosynthesis and leaf conductance than a low rate of water stress in sorghum. Stuart et al. (1985) reported that stomatal movement limited the photosynthesis of water-stressed Johnsongrass, and there was a strong correlation between decreasing osmotic potential and photosynthesis.

Aronson et al. (1987) compared the soil water potentials at which the evapotranspiration of cool season turfgrass species declined, and found that *Festuca* species had the best capability to thrive at low soil water content. Ghashghaie and Saugier (1989) provided valuable information about the photosynthesis and stomatal movement of tall fescue subjected to drought and different nitrogen levels at the same time. Johnson et al. (1987) observed the photosynthesis and conductance of *Triticum* species over a soil drying cycle. Jones et al. (1980) described the stomatal behaviour and morphological changes of perennial ryegrass subjected to slow and rapid rates of water stress. The authors found a discrete value of leaf water potential when the leaf resistance of ryegrass started to increase, but different rates of water stress did not cause a difference in stomatal behaviour.

Under field conditions grasses undergo several moisture stress cycles, which can lead to changes in water stress response. Jones and Rawson (1979) found that slight water stress did not significantly influence the physiological responses to the succeeding moisture stress cycle. King and Bush (1985) compared the growth, osmotic potential and leaf water potential changes of previously water stress-conditioned and non-water stress-conditioned tall fescue over a decreasing range of soil water contents. Conditioned plants showed a higher elongation rate than non-conditioned plants, but there was no difference in the osmotic and leaf water potentials. Seiler and Johnson (1988a, b) reported that water stress conditioning made loblolly pine seedlings tolerant of moisture deficiency and capable of maintaining turgor to lower needle potential than non-conditioned seedlings. They found increased drought tolerance in conditioned seedlings, since the photosynthesis of conditioned plants ceased at lower leaf water potential than that of non-conditioned plants. The transpiration of conditioned seedlings was reduced, while water use efficiency increased.

The purpose of this experiment was to investigate the influence of water stress conditioning on the drought responses of a warm season grass (switchgrass) and a cool season grass (tall fescue).

Materials and methods

Plant material and growing conditions

The Pathfinder cultivar of switchgrass (*Panicum virgatum* L.) and the Kentucky 31 cultivar of tall fescue (*Festuca arundinacea* Schreb.) were transplanted from vigorous field stands to pots containing a mixture of clay-loam soil and sand at a ratio of 2:1. The soil/sand mixture had a pH of 5.6, a phosphorus content of 48 ppm and a potassium content of 96 ppm. Immediately after transplantation 1.07 g of Peter's solution (Peters General Purpose, Fogelsville, PA), with 20% nitrogen, phosphorus and potassium content, was applied as fertilizer to each pot. The plants were grown in a growth chamber, where the average temperature was 25°C, average relative humidity was 70%, photosynthetically active radiation was 370 E/m^2 and the photoperiod was 12 h. The tillers had an average height of 20 cm and 2 to 3 leaves with exposed collars at the beginning of the experiment.

Treatments

Two soil water treatments were assigned to pots of each species.

– Non-conditioned plants were kept well watered for 21 days.

– Water was withheld from the soil of the pots assigned to the conditioning treatment to impose moisture deficits. The elongation of one tiller per pot was measured daily during soil water content decline. When the growth of the plants stopped, the conditioning cycle ceased and the pots were rewatered approximately to soil saturation. The water stress cycle was repeated three times, to induce the water stress conditioning of the plants.

At the end of the conditioning treatment water was withheld from all the pots.

Measurements

The elongation of one tiller per pot and the soil water content of the pots were monitored every second day during the conditioning treatment. The length of the leaves was measured from a standard point on the pot until two successive measurements were the same. The total elongation of the tillers was calculated as the sum of all the leaf elongations. The soil water content of the pots was established by means of gravimetric measurements according to the following equation:

$$\mathrm{SWC} = 100\,\frac{\mathrm{MW{-}DW}}{\mathrm{DW}}$$

where SWC is soil water content, MW is the moist weight of the soil in the pot, and DW is the dry weight of soil in the pot.

The leaf water potential and photosynthesis of the plants were measured daily at 4 pm. during the last moisture deficiency cycle. The leaf water potential of the flag leaf from one tiller was determined using a pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA) as described by Kirkham (1985). For gas exchange measurement a Licor 6200 portable infrared CO_2 analyser with an open system (Licor, Lincoln, NE) was used. The photosynthesis at the same position on the flag leaf on an assigned tiller in each pot was observed. The environmental conditions during the measurements were: temperature 28.8±0.8°C, photosynthetic photon flux density 347±32 E/m^2, CO_2 concentration 352±12 ppm, relative humidity 34±5%. Photosynthesis was determined until the measurements decreased to near zero for a group of species and treatments.

Leaf conductance and transpiration were recorded during gas exchange measurements, when both conditioned and non-conditioned switchgrass and tall fescue were subjected to moisture deficiency.

The mesophyll resistance to CO_2 transfer was calculated using simultaneously determined photosynthesis and internal CO_2 concentration data (Osonubi and Davies, 1980) according to the following equation:

$$r_m = \frac{C_i}{Ps}$$

where r_m is mesophyll resistance, C_i is internal CO_2 concentration, and Ps is the photosynthesis rate.

Results

Soil water content and elongation during water stress conditioning

Non-conditioned switchgrass pots were kept well watered with an average soil water content of 26.4±1.7%, while the non-conditioned tall fescue pots had an average soil water content of 21.5±3.1%. During the conditioning moisture deficiency cycles the soil water content of the switchgrass pots decreased by 20.7% and that of the tall fescue pots by 13.7%. The difference in the extent to which the soil water content decreased in the tall fescue and switchgrass pots is due to the difference in leaf area above the pots.

The decrease in elongation during conditioning was independent of species differences, as also reported by Coyne et al. (1982) (Table 1). Switchgrass tillers had 65.3% more elongation than tall fescue tillers and non-conditioned plants had 78.2% more elongation than conditioned plants. The changes in soil water content and elongation during water stress conditioning determined the length of conditioning water stress cycles.

Leaf water potential at zero photosynthesis and elongation

The leaf water potential of conditioned and non-conditioned tillers was compared when the photosynthesis and elongation of switchgrass and tall fescue became zero in the last water stress cycle (Table 2).

Photosynthesis ceased at a lower leaf water potential than elongation. Species and treatment had an influence on the number of days required for photosynthesis to become zero. The photosynthesis of non-conditioned tall fescue stopped in 4 days, while the photosynthesis of conditioned tall fescue continued for 6 days. Non-conditioned switchgrass photosynthesized for 6 days and conditioned switchgrass for 8 days. Conditioning and species differences affected the leaf water potential at zero photosynthesis independently of each other. The leaf water potential of conditioned plants was 44% lower than that of non-conditioned plants when photosynthesis ceased.

Table 1

Total elongation (mm) of water stress-conditioned and non-water stress-conditioned switchgrass and tall fescue during conditioning

Treatments	Species		Average
	Switchgrass	Tall fescue	
Conditioned	442	269	363a*
Non-conditioned	887	467	647b
Average	590a	357b	

*Numbers followed by the same letter within row or column are not significantly different at P=0.05 by the LSD test.

Table 2

Leaf water potential (Ψ; MPa) when elongation and photosynthesis of water stress-conditioned and non-water-stress conditioned switchgrass and tall fescue became zero during water withholding

Treatments	Species		Average
	Switchgrass	Tall fescue	
		φ when elongation is zero	
Conditioned	2.37	3.18	2.82a*
Non-conditioned	1.84	2.69	2.32b
Average	2.14a	2.96b	
		φ when photosynthesis is zero	
Conditioned	4.35	3.93	4.16a
Nonconditioned	3.04	2.69	2.88b
Average	3.76a	3.38b	

*Numbers followed by the same letter within row or column are not significantly different at P=0.05 by the LSD test.

Switchgrass stopped photosynthesizing at 11% lower leaf water potential than tall fescue.

These results suggest that conditioning improves the photosynthetic capacity of the plants. The magnitude of the changes in leaf water potential, induced by conditioning at zero photosynthesis, is the same for different species. However, switchgrass plants continued photosynthesizing to a lower leaf water potential whether they were conditioned or not. This result was predictable, because switchgrass is a grass of tropical origin, with a C_4 photosynthesis pattern. Probably an even higher difference between switchgrass and tall fescue could be observed if the photosynthetically active radiation level were higher than during this experiment.

The influence of conditioning on the leaf water potential at zero elongation was dependent on the species. The elongation of conditioned switchgrass became zero at 28% lower leaf water potential than that of non-conditioned switchgrass. The elongation of conditioned tall fescue became zero at 18% lower leaf water potential than that of non-conditioned tall fescue. The elongation of tall fescue stopped at lower leaf water potential than that of switchgrass. Based on the leaf water potential values described above at zero photosynthesis and data in the literature (e.g. Stout et al., 1986), one would expect switchgrass to stop elongating at a lower leaf water potential than tall fescue. The reason for this unexpectedly high leaf water potential at zero elongation for switchgrass was that the tillers began to develop inflorescences during the experiment.

Photosynthesis decrease during moisture deficiency

The change in photosynthesis over a decreasing range of leaf water potential is shown in Fig. 1. Photosynthesis measurements were taken in the range –0.8 to –4.0 MPa leaf water potential, when the withholding of water caused a mean water stress rate of 0.49 MPa/day. The photosynthesis of switchgrass and tall fescue during a drought cycle exhibited a linear relationship with leaf water potential and this linear relationship was different whether the plants were water stress conditioned or not.

Ghashghaie and Saugier (1989) found a similar linear relationship between the same range of leaf water potential and photosynthesis in tall fescue with a low nitrogen supply. Beadle et al. (1973) investigated the photosynthesis of maize and sorghum as influenced by declining leaf water potentials between –0.2 and –1.6 MPa. They observed a linear decrease in photosynthesis when the leaf water potential declined to less than –1.1 MPa. Johnson et al. (1987) investigated the photosynthesis response of *Triticum* species and found a curvilinear relationship between water potential and photosynthesis. Based on these observations it was concluded that the data showed the linear part of a curvilinear relationship, so the intercept data were not used as the maximum value of photosynthesis.

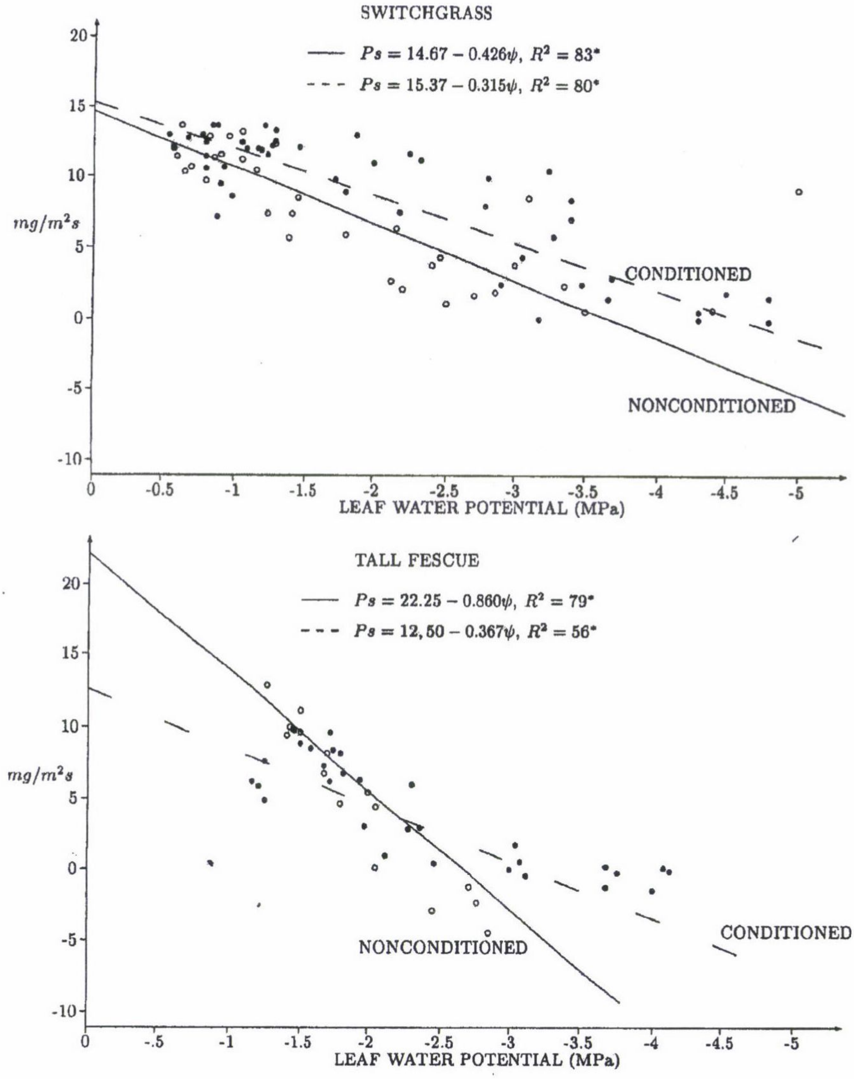

Fig. 1. Photosynthesis during water stress of conditioned and non-conditioned tall fescue and switchgrass plants. (Slopes of conditioned and non-conditioned plants for both switchgrass and tall fescue are significantly different at the 5% level of significance.)

Comparing the species, the decrease in the photosynthesis of non-conditioned tall fescue and switchgrass during the drought cycle was statistically different. The photosynthesis of tall fescue declined by a rate 102% greater than that of switchgrass. On the other hand, the decrease in the photosynthesis of conditioned plants was not statistically different. Although the photosynthesis of switchgrass improved after water stress conditioning, tall fescue benefited much more than switchgrass.

Seiler and Johnson (1988a) concluded that the influence of conditioning on the photosynthesis response to water stress in loblolly pine was closely releated to the number of conditioning water stress cycles. In the present study switchgrass and tall fescue were subjected to the same drought conditioning treatment and the acclimation of both species resulted in the same rate of photosynthesis decrease while withholding water.

The photosynthesis of water stress-conditioned and non-water stress-conditioned plants was significantly different during moisture deficiency. The photosynthesis of conditioned switchgrass plants was 26% more than that of non-conditioned switchgrass. Conditioned tall fescue plants photosynthesized 57% more than non-conditioned tall fescue plants.

Leaf conductance, transpiration and mesophyll resistance

The leaf conductance, transpiration and mesophyll resistance of plants upon rewatering after three conditioning drought cycles were calculated to investigate the role of the stomata in the photosynthesis response to water stress (Table 3).

Table 3

Leaf water potential (Ψ), photosynthesis (Ps), stomatal conductance (Cs), mesophyll resistance (r_m) and transpiration (Tr) of water stress-conditioned and non water-stress-conditioned switchgrass and tall fescue on the first day and switchgrass on the third day of water withholding

Treatments	φ	Ps	Cs	r_m	Tr
	MPa	g/cm^{2}s	g/cm^{2}s	s/cm	g/cm^{2}s
		Tall fescue on the first day			
Conditioned	1.26a*	6.9a	0.524a	12.78a	0.0046a
Non-conditioned	1.38a	10.5b	1.165b	18.69b	0.0080b
		Switchgrass on the first day			
Conditioned	0.84a	9.9a	0.172a	2.54a	0.0017a
Non-conditioned	0.64b	11.6a	0.191a	3.15a	0.0019a
		Switchgrass on the third day			
Conditioned	1.00a	12.6a	0.316a	6.81a	0.0031a
Non-conditioned	1.10a	10.2b	0.247b	5.40a	0.0025a

*Numbers followed by the same letter within row or column are not significantly different at the P=0.05 level by the LSD test.

The conditioning of tall fescue reduced leaf conductance by 55%, mesophyll resistance by 32% and transpiration by 42%. The leaf water potential of tall fescue recovered fully one day after rewatering following the conditioning cycles. The significant decrease in leaf conductance as the result of conditioning could be the reason for reduced transpiration and photosynthetic rate and for the quick recovery of leaf water potential. The lower mesophyll resistance of conditioned plants suggests that either the diffusion of CO_2 to mesophyll cells or the carboxylation efficiency improved. However, this reduction in mesophyll resistance was unable to improve the photosynthetic rate on the first day after water stress conditioning.

Water stress conditioning did not influence the leaf conductance, mesophyll resistance or transpiration of switchgrass. The leaf water potential of conditioned switchgrass plants did not recover, but was significantly lower than that of non-conditioned switchgrass one day after rewatering, following the conditioning cycles. On the other hand, both conditioned and non-conditioned tillers had the same photosynthetic rate on the same day. Additionally, conditioned plants showed the same leaf conductance, transpiration and mesophyll resistance at a lower leaf water potential than non-conditioned plants. On the third day, when the leaf water potential of conditioned and non-conditioned tillers became equal, the photosynthesis of conditioned plants was significantly higher, due to higher leaf conductance level, than that of non-conditioned plants.

The transpiration of conditioned and non-conditioned switchgrass was equal despite the increased leaf conductance of conditioned tillers. There was no difference in the mesophyll resistance of conditioned and non-conditioned switchgrass, which suggests that neither the CO_2 diffusion to mesophyll cells nor the carboxylase activity of PEP carboxylase was affected by the conditioning treatment.

Discussion

Water stress conditioning gave protection against some loss of photosynthesis in tall fescue, a cool season grass, and in switchgrass, a warm season grass, during moisture deficiency. Both plant species acclimated their photosynthesis rate to the same level during the drought cycles. Conditioned plants were able to maintain their photosynthesis and elongation down to lower leaf water potentials than non-conditioned plants during moisture deficiency. The beneficial results of water stress conditioning were costly. During conditioning, the elongation of the leaves was significantly reduced compared to that of non-conditioned plants, but after rewatering there was a compensating photosynthetic advantage.

The conclusion of this study to researchers is that when evaluating the physiological water stress responses of plants, one should be aware of the water conditions before the observations are taken. It is especially important to know the prior water stress history in field experiments.

This study could also help farmers to grow tall fescue and switchgrass and to use irrigation effectively. Tall fescue tillers become dormant during the driest days of summer and give their best yield in the spring and early summer. If switchgrass is available during the summer slump, the best irrigation management could be to water tall fescue and avoid the elongation decrease caused by drought stress, but to allow switchgrass plants to undergo moisture deficiency cycles that improve their photosynthetic response, therefore yield response, to drought.

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SALT STRESS RESPONSE OF SALT-SENSITIVE AND TOLERANT DURUM WHEAT CULTIVARS INOCULATED WITH MYCORRHIZAL FUNGI

G. N. AL-KARAKI

FACULTY OF AGRICULTURE, JORDAN UNIV. OF SCIENCE AND TECHNOLOGY, IRBID, JORDAN

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The effects of arbuscular mycorrhizal fungi (AMF) and salt stress on growth and nutrient acquisition in two durum wheat (*Triticum durum* Desf.) cultivars exhibiting differences in salt tolerance were investigated. The plants were grown in a sterilized, low P (silty clay) soil-sand mix. Three salt levels were created by adding NaCl solution to the soil through irrigation water, resulting in saturation extract (EC_e) values of 1.2 (control), 4.1 (medium) and 6.7 dS m^{-1} (high salt stress), respectively. Mycorrhizal colonization occurred whether the soil was salt stressed or non-stressed and in both cultivars, but the extent of AMF colonization was higher in the control than under saline soil conditions. The salt-tolerant cultivar Petra had higher mycorrhizal colonization than the salt-sensitive cultivar Hourani-27. The shoot dry matter (DM) yield was higher in mycorrhizal than in non-mycorrhizal plants of both cultivars. Petra had higher shoot DM but not higher root DM than Hourani-27 plants. The enhancement in shoot DM due to AMF inoculation was 22 and 21% in the control, 31 and 58% at medium, and 18 and 60% at high salinity level for Petra and Hourani-27, respectively. For both cultivars, the contents of P, K, Zn, Cu and Fe were higher in mycorrhizal than in non-mycorrhizal plants under control and medium saline soil conditions. Shoot Na concentrations were lower in mycorrhizal than in non-mycorrhizal plants grown under saline conditions. The enhancement in P, K, Zn, Cu and Fe acquisition due to AMF inoculation was more pronounced in Hourani-27 than in Petra under saline soil conditions. The results suggest that Hourani-27 tends to benefit from AMF colonization more than Petra under saline soil conditions, despite the fact that Petra roots were highly colonized with the AM fungus. It appears that Hourani-27 is more dependent on AMF symbiosis than Petra.

Key words: *Triticum durum*, mycorrhiza, salt stress

Introduction

One of the most serious agricultural problems in arid and semiarid regions is the accumulation of salt on the soil surface, which renders the field unproductive. In general, salinity inhibits plant growth and productivity. The detrimental effects of salinity on plant growth result from the direct effects of ion toxicity (Al-Karaki, 2000a; Ayers and Westcot, 1985; Hasegawa et al., 1986) or the indirect effects of saline ions that cause soil/plant osmotic imbalance (Wyn Jones and Gorham, 1983). Using factors which enable plants to withstand salt stress better would help to improve crop production under saline conditions.

The introduction of arbuscular mycorrhizal fungi (AMF) to sites with saline soil may improve plant tolerance and growth (Al-Karaki, 2000b; Jain et al., 1989). The improved productivity of AMF plants has been attributed especially to the enhanced acquisition of less mobile nutrients such as P, Zn and Cu (Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark, 1998; George et al., 1994; Marschner and Dell, 1994) and improved water relations (Al-Karaki, 1998; Bethlenfalvay et al., 1988; Sylvia et al., 1993). Not only do mycorrhizal associations with plant roots enhance growth and mineral element uptake, but mycorrhizal plants may have greater tolerance to salt stress (Al-Karaki, 2000b; Ruiz-Lozano et al., 1996). This improved salt tolerance following mycorrhizal colonization could be caused by more efficient P uptake by mycorrhizal plants in P deficient soils (Poss et al., 1985), leading to increased growth and the subsequent dilution of toxic ion effects (Juniper and Abbott, 1993). However, some researchers have reported that the salt tolerance of AMF plants is independent of the plant P concentration (Ruiz-Lozano et al., 1996; Danneberg et al., 1992).

Salinity tolerance by wheat (*Triticum* spp.) plants is a major concern in Mediterranean regions, where plants are often subjected to high levels of salinity in the soil from soluble salts in irrigation water and fertilizers, due to the negative correlation between excess salinity and yield (Abu Samra, 1999; Francois et al., 1986; Maas and Poss, 1989). A wide variation in plant responses to AMF inoculation has been reported for wheat and other plant species under environmental stresses (Al-Karaki and Al-Raddad, 1997; Ellis et al., 1985; Kwapata and Hall, 1985). It has been suggested that mycorrhizal colonization is a host-dependent and heritable trait (Lackie et al., 1988; Mercy et al., 1990).

To determine whether mycorrhizal colonization influences host plant responses to salt stress, growth parameters and mineral nutrient acquisition under different levels of salt stress were monitored in durum wheat cultivars differing in salt tolerance.

Materials and methods

A greenhouse experiment was conducted at 26±4°C under natural illumination during the spring of 1999. Wheat plants were grown in a silty clay soil (fine, mixed, thermic, Typic Xerochrept) mixed with sand [soil:sand, 2:1 (v/v)]. The soil properties before mixing with sand were 6.5% sand, 45% silt, and 48.5% clay; 1.2% organic matter; pH 8.1 (soil:water, 1:1); electrical conductivity (ECe) 1.1 dS m^{-1}; 0.26 P ($NaHCO_3$-extractable), 23.1 K, 6.2 Na, 0.2 Fe, 0.02 Zn and 0.03 Cu (5 mM DTPA-extractable) in mmol kg^{-1} soil. The soil mix was heat pasteurized (100°C) for 3 h, left to stand at ambient temperature overnight, and subjected to further pasteurization at 100°C for 3 h. The soil mix was dispensed into plastic pots (4.5 kg soil per pot) for plant growth. No P was added to the soil.

Half of the pots received the AMF *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe by placing 40 g (moist weight) of inoculum in the soil at a depth of 3 cm in 10 cm diameter holes in the centre of the pots prior to planting. The AMF inoculum added to the pots consisted of soil and root fragments and spores (1080 chlamydospores kg^{-1} air-dried soil). The mycorrhizal inoculum (*Glomus mosseae*) was initially isolated from a wheat (*Triticum durum* Desf.) field in northern Jordan (Al-Raddad, 1993) and multiplied in pot cultures using chickpea (*Cicer arietinum* L.) as a host (Al-Karaki and Al-Raddad, 1997). Control treatments received no AMF inoculum.

Seeds of the durum wheat cultivars Petra (salt-tolerant) and Hourani-27 (salt-sensitive) (Abu Samra, 1999) were planted near the centre of each pot. One week after emergence, the wheat seedlings were thinned to four per pot. Nitrogen was added at a rate of 30 mg N kg^{-1} soil as NH_4NO_3 7 days after seedling emergence. The plants were watered for three weeks, after which they were subjected to three salt stress levels. Salt stress was imposed by adding NaCl solution to the soil through the irrigation water, resulting in saturation extract (ECe) values of 1.2 (control), 4.1 (medium), and 6.7 (high salt stress) dS m^{-1}, respectively. The soil was salinized step-wise to avoid osmotic shock. The plants were then watered with tap water (EC= 0.3 dS m^{-1}) until they were harvested. When leaching occurred, the leachate was collected and added to the soil to maintain the salinity treatments near the target levels.

The experiment was terminated by severing the shoots from the roots after the plants had been grown under salt stress conditions for eight weeks. The shoots were then oven-dried at 70°C for 48 h, weighed and saved for mineral analysis. The roots were rinsed free from soil, cut into 1 cm fragments, and thoroughly mixed, after which representative fresh samples (1 g) were removed for the determination of root AMF colonization. The remaining roots were dried and weighed. Root samples for the determination of root colonization with AMF were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol, as described by Phillips and Hayman (1970), and examined microscopically for AMF colonization. The percentage of root segments containing arbuscules + vesicles was determined using a gridline intercept method (Bierman and Linderman, 1981).

Dried shoots were ground to pass through a 0.5 mm sieve in a cyclone laboratory mill, and saved for the determination of mineral nutrients. The shoot P concentration was determined colorimetrically (Watanabe and Olsen, 1965) and the Zn, Fe and Cu concentrations by atomic absorption spectroscopy. The K and Na concentrations in the plant shoots were determined using a flame photometer.

The experiment was randomized in complete blocks with three salt stress levels, two AMF inoculum treatments and two wheat cultivars to give a 3 × 2 × 2 factorial with four replications. The data were statistically analysed by analysis of variance using the MSTATC program (Michigan State Univ., East Lansing, MI). Probabilities of significance among treatments and interactions and LSDs ($P < 0.05$) were used to compare means within and between treatments. Mean percentages of AMF colonization were calculated from arcsine transformed data.

Results

Nearly all salinity and AMF treatment effects were significant for the growth and nutrient acquisition traits (Table 1). Salt × AMF interactions were significant for shoot and root dry matter (DM) yields, AMF colonization, P and Na concentrations and P and Fe contents. The cultivar effect was significant for shoot DM, AMF colonization, P, K and Na concentrations, and P, K and Fe contents. Significant interactions involving the cultivar were salt × C, AMF × C and salt × AMF × C for Na concentration and AMF × C for AMF colonization (Table 1).

No AMF colonization was noted in the roots of control plants. Wheat plants grown in non-saline soil had relatively high AMF root colonization (~45%), which decreased as the soil salinity increased (Table 2). Under non-saline (1.2 dS m^{-1}) and highly saline (6.7 dS m^{-1}) conditions the roots of the salt-tolerant cultivar Petra showed significantly higher AMF colonization than the roots of the salt-sensitive cultivar Hourani-27, but not under moderate (4.1 dS m^{-1}) salt conditions (Table 2).

Wheat shoot and root DM were higher for mycorrhizal than for non-mycorrhizal plants (Table 2). However, AMF inoculation had no significant effect on shoot DM in the high salinity treatment or on root DM in the non-saline and medium salinity treatments. Shoot and root DM decreased as soil salinity increased in both mycorrhizal and non-mycorrhizal plants (Table 2). Petra had higher shoot DM than Hourani-27, but these differences were significant only in non-mycorrhizal plants at all salinity levels. No significant differences in shoot and root DM between the cultivars occurred after AMF inoculation (Table 2).

Table 1
Probabilities of significance for different growth and mineral acquisition traits in wheat cultivars grown under different salinity levels and inoculated or not with AMF

Trait	Salt level	AMF status	Cultivar (C)	Salt×AMF	Salt×C	AMF×C	Salt×AMF×C
Shoot DM	**	**	**	**	NS	NS	NS
Root DM	**	**	NS	**	NS	NS	NS
AMF colonization	**	**	**	**	NS	**	NS
P concentration	**	**	**	**	NS	NS	NS
P content	**	**	**	**	NS	NS	NS
K concentration	**	**	*	NS	NS	NS	NS
K content	**	**	**	NS	NS	NS	NS
Na concentration	**	**	**	**	*	**	*
Na content	**	NS	NS	NS	NS	NS	NS
Cu concentration	**	**	NS	NS	NS	NS	NS
Cu content	**	**	NS	NS	NS	NS	NS
Fe concentration	**	**	NS	NS	NS	NS	NS
Fe content	**	**	*	*	NS	NS	NS
Zn concentration	**	**	NS	NS	NS	NS	NS
Zn content	**	**	NS	NS	NS	NS	NS

* , ** Significant at $P<0.05$ and $P<0.01$, respectively; NS: not significant

Table 2
Root AMF, shoot and root dry matter yields for non-mycorrhizal (NonAMF) and mycorrhizal (AMF) wheat cultivars grown at different salinity levels

Salt level dS m^{-1}	AMF status	Cultivar	AMF colonization	Dry matter (g plant^{-1})	
			%	Shoot	Root
1.2	NonAMF	Petra	0.0	4.43	0.47
		Hourani-27	0.0	4.13	0.44
	AMF	Petra	49.5	5.39	0.89
		Hourani-27	40.2	4.99	0.90
4.1	NonAMF	Petra	0.0	3.06	0.33
		Hourani-27	0.0	2.33	0.32
	AMF	Petra	38.8	4.02	0.44
		Hourani-27	34.6	3.68	0.36
6.7	NonAMF	Petra	0.0	1.56	0.10
		Hourani-27	0.0	1.09	0.07
	AMF	Petra	30.4	1.84	0.30
		Hourani-27	24.9	1.74	0.15
LSD (0.05)			4.0	0.41	0.20

Shoot P concentrations and contents were higher in mycorrhizal than in non-mycorrhizal wheat plants of both cultivars regardless of the salinity level (Tables 3 and 4). However, no significant differences were noted for shoot P contents between mycorrhizal and non-mycorrhizal plants of the cultivar Petra at the high salinity level. Shoot P concentrations and contents decreased with increasing soil salinity in both mycorrhizal and non-mycorrhizal plants (Tables 3 and 4). Petra had higher shoot P concentrations than Hourani-27 in non-mycorrhizal plants but not mycorrhizal plants at the medium and high salinity levels (Table 3). No significant differences in P concentration due to AMF inoculation were noted between the cultivars at either salinity level. Genotypic differences in P content due to AMF inoculation were noted only under non-saline conditions, when Petra had higher shoot P contents than Hourani-27. However, Petra had higher shoot P contents than Hourani-27 for non-mycorrhizal plants at the moderate salinity level.

Shoot K concentrations were similar for mycorrhizal and non-mycorrhizal plants of both cultivars except for Hourani-27 at the medium salinity level (Table 3). Shoot K contents were higher in mycorrhizal than in non-mycorrhizal plants for both cultivars in the non-saline and medium salinity treatments. Shoot K concentrations and contents decreased as soil salinity increased (Tables 3 and 4). No significant differences were noted between the cultivars for shoot K concentrations. Petra had higher shoot K contents than Hourani-27 in mycorrhizal plants in the non-saline treatment and in non-mycorrhizal plants in the medium salinity treatment (Table 4).

Shoot Na concentrations and contents of both mycorrhizal and non-mycorrhizal plants increased as soil salinity increased, but the differences for Na contents were only significant as salinity increased from the non-saline to the medium level (Tables 3 and 4). Sodium concentrations were lower in mycorrhizal than in non-mycorrhizal plants of both cultivars at medium and high salinity levels, but not in the non-saline treatment (Table 3). No significant differences were noted between the cultivars due to AMF inoculation for Na concentrations and contents regardless of the salinity level, though Hourani-27 had higher Na concentrations but not contents than Petra for non-mycorrhizal plants at the medium and high salinity levels (Tables 3 and 4).

Table 3

Shoot concentrations of P, K and Na in non-mycorrhizal (NonAMF) and mycorrhizal (AMF) wheat cultivars grown at different salinity levels

Salt level dS m^{-1}	AMF status	Cultivar	Shoot concentration (mg g^{-1} DM)		
			P	K	Na
1.2	NonAMF	Petra	0.96	38	3.7
		Hourani-27	0.89	37	4.0
	AMF	Petra	1.57	41	3.2
		Hourani-27	1.48	38	3.4
4.1	NonAMF	Petra	0.71	32	24.2
		Hourani-27	0.58	27	29.9
	AMF	Petra	1.42	34	16.1
		Hourani-27	1.37	34	15.9
6.7	NonAMF	Petra	0.58	23	42.2
		Hourani-27	0.43	20	59.0
	AMF	Petra	0.86	25	32.7
		Hourani-27	0.74	24	34.1
LSD (0.05)			0.13	6	5.7

Table 4
Shoot contents of P, K and Na in non-mycorrhizal (NonAMF) and mycorrhizal (AMF) wheat cultivars grown at different salinity levels

Salt level dS m^{-1}	AMF status	Cultivar	Shoot content (mg $plant^{-1}$)		
			P	K	Na
1.2	NonAMF	Petra	4.24	168	16.6
		Hourani-27	3.70	152	16.6
	AMF	Petra	8.51	224	16.7
		Hourani-27	7.40	189	16.8
4.1	NonAMF	Petra	2.18	99	73.2
		Hourani-27	1.37	63	69.9
	AMF	Petra	5.71	138	64.4
		Hourani-27	5.03	124	58.3
6.7	NonAMF	Petra	0.90	36	65.7
		Hourani-27	0.49	22	64.4
	AMF	Petra	1.59	47	60.0
		Hourani-27	1.30	41	58.7
LSD (0.05)			0.77	34	13.2

The shoot concentrations of Cu and Fe were generally higher for mycorrhizal than for non-mycorrhizal plants at all salinity levels, though the differences were not significant for Cu at the high salinity level or for Fe at the medium and high salinity levels (Table 5).

Table 5
Shoot concentrations of Cu, Fe and Zn in non-mycorrhizal (NonAMF) and mycorrhizal (AMF) wheat cultivars grown at different salinity levels

Salt level dS m^{-1}	AMF status	Cultivar	Shoot concentration (μg g^{-1} DM)		
			Cu	Fe	Zn
1.2	NonAMF	Petra	12.1	136	45
		Hourani-27	11.5	132	44
	AMF	Petra	15.4	185	54
		Hourani-27	14.0	179	51
4.1	NonAMF	Petra	6.6	131	29
		Hourani-27	4.9	129	27
	AMF	Petra	12.2	152	34
		Hourani-27	12.2	149	33
6.7	NonAMF	Petra	4.8	111	24
		Hourani-27	3.5	109	23
	AMF	Petra	10.2	144	32
		Hourani-27	7.8	142	31
LSD (0.05)			4.5	37	10

Shoot Zn concentrations were similar for mycorrhizal and non-mycorrhizal plants of both cultivars grown at all salinity levels. Shoot contents of Cu, Fe and Zn were generally higher for mycorrhizal than for non-mycorrhizal plants, but these differences were not significant for Cu and Fe at the high salinity level or for Zn at the medium and high salinity levels (Table 6). Shoot concentrations and contents of Cu, Fe and Zn decreased as soil salinity increased. No significant differences were noted between the cultivars for shoot concentrations and contents of Cu, Fe and Zn in either mycorrhizal or non-mycorrhizal plants.

The overall effects of AMF colonization on the shoot dry matter yield and mineral nutrient acquisition of saline and non-saline plants are summarized in Table 7.

Table 6

Shoot contents of Cu, Fe and Zn in non-mycorrhizal (NonAMF) and mycorrhizal (AMF) wheat cultivars grown at different salinity levels

Salt level dS m^{-1}	AMF status	Cultivar	Shoot content (μg $plant^{-1}$)		
			Cu	Fe	Zn
1.2	NonAMF	Petra	52.8	609	197
		Hourani-27	47.9	548	180
	AMF	Petra	84.0	999	295
		Hourani-27	70.0	895	257
4.1	NonAMF	Petra	20.7	403	88
		Hourani-27	11.5	302	64
	AMF	Petra	49.2	611	136
		Hourani-27	44.3	546	121
6.7	NonAMF	Petra	7.5	174	38
		Hourani-27	3.8	121	25
	AMF	Petra	19.1	270	59
		Hourani-27	13.8	249	55
LSD (0.05)			20.2	168	55

Table 7

Percentage of calculated mycorrhizal (AMF) increases/decreases in shoot dry matter (DM) yield and mineral contents in wheat cultivars grown at different salinity levels

Salt level dS m^{-1}	Cultivar	Shoot DM*	Mineral content** (%)					
			P	K	Na	Cu	Fe	Zn
1.2	Petra	22	100	33	1	59	64	50
	Hourani-27	21	100	25	1	46	63	43
4.1	Petra	31	162	40	−12	137	52	54
	Hourani-27	58	266	95	17	286	81	88
6.7	Petra	18	77	29	9	155	55	53
	Hourani-27	60	165	87	9	260	106	119

*Shoot DM increase = $DM_{AMF} - DM_{nonAMF} \times 100 / DM_{nonAMF}$; **Mineral content (MC) increase/decrease = $MC_{AMF} - MC_{nonAMF} \times 100 / MC_{nonAMF}$

Discussion

Plants inoculated with mycorrhizal fungi had higher shoot and root DM yields than non-mycorrhizal plants at all salinities, but the responses were only significant at medium salinity (4.1 dS m^{-1}) and under non-saline conditions for shoot DM and under non-saline conditions for root DM. The enhanced growth of mycorrhizal plants when grown in saline environments has been attributed partly to the mycorrhiza-mediated enhancement of host plant P nutrition (Al-Karaki, 2000b; Hirrel and Gerdemann, 1980; Pond et al., 1984; Poss et al., 1985). In this study, mycorrhizal plants had higher P concentrations and contents than non-mycorrhizal plants at all salinity levels, but the differences for P contents were not significant at the high salinity level. The plant growth enhancement attributed to AMF root colonization decreased in the high salinity (6.7 dS m^{-1}) treatment. This might have occurred because of reduced P transport and uptake under these conditions. Plants grown under high salinity may have lower $H_2PO_4^-$ activity (preferred phosphate ion for plant uptake) than when grown under low salinity conditions (Al-Karaki, 1997; Sentenac and Grignon, 1985). Reduced uptake of P by mycorrhizal plants grown at high salinity levels was also reported by other workers (Al-Karaki, 2000b; Hirrel and Gerdemann, 1980; Pond et al., 1984; Poss et al., 1985).

Many studies have indicated that AMF contributes to plant growth via the enhancement of mineral nutrient uptake, especially that of immobile soil nutrients (P, Cu, Zn) (Bethlenfalvay et al., 1988; Marschner and Dell, 1994; Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark, 1998). In this study, the mycorrhizal wheat plants had higher shoot P concentrations and contents than the non-mycorrhizal plants regardless of salinity level. Higher Fe and Cu concentrations and contents were also noted in mycorrhizal plants compared to non-mycorrhizal plants. The higher mineral nutrient acquisition in mycorrhizal plants compared to non-mycorrhizal plants may have occurred because of increased availabilities or transport (absorption and/or translocation) by AMF hyphae. The enhanced acquisition of P, Cu and Fe by mycorrhizal plants has been reported (Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark, 1998; Marschner and Dell, 1994; Trimble and Knowles, 1995). However, AMF root colonization did not significantly affect shoot K concentrations in plants grown at any of the salinity levels. Poss et al. (1985) reported that K uptake was affected little by AMF root colonization in tomato (*Lycopersicon esculentum* Mill.) grown under salinity conditions.

Shoot Na concentrations but not contents were lower in mycorrhizal than in non-mycorrhizal plants regardless of salinity level. The lack of response of Na contents to AMF treatment might be explained by dilution effects due to plant growth enhancement caused by AMF colonization. Similar results were reported by other researchers (Bernstein et al., 1974; Jarrell and Beverly, 1981).

Plant growth responses to AMF inoculation were higher in Hourani-27 than in Petra under saline but not under non-saline conditions, even though AMF colonization was higher in Petra than in Hourani-27. However, in the presence of AMF, the differences between Petra and Hourani-27 in root dry matter were not significant. Enhanced growth may not be related to the degree of AMF root colonization in wheat (Al-Karaki and Clark, 1998).

The host plant species, cultivar and growing conditions may influence the effectiveness of AMF symbiosis in nutrient acquisition (Al-Karaki, 2000b; Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark, 1998; Mercy et al., 1990). From the results of this study, it appears that AMF colonization was more effective in increasing P, Cu, Fe and Zn acquisition under saline conditions for the salt-sensitive cultivar Hourani-27 than the salt-tolerant cultivar Petra. Greater nutrient acquisition in response to AMF colonization was suggested to be a plant strategy for salt stress tolerance (Hirrel and Gerdemann, 1980; Pond et al., 1984; Poss et al., 1985).

The improved growth and nutrient acquisition in mycorrhizal wheat demonstrate the potential of AMF colonization for the protection from salt stress of plants grown in arid and semiarid regions. However, several AMF isolates should be investigated in order to maximize the efficiency of AMF symbiosis under saline conditions.

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CALCIUM ENHANCEMENT OF SHOOT ORGANOGENESIS IN SALINITY-STRESSED TOMATO EXPLANTS

A. E. EL-ENANY, A. A. ISSA and R. ABDEL-BASSET

BOTANY DEPARTMENT, FACULTY OF SCIENCE, UNIVERSITY OF ASSIUT, ASSIUT, EGYPT

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Efficient *de novo* shoot organogenesis from hypocotyl and cotyledons was studied under NaCl-salinity conditions and in a salinity-calcium combination. Sodium chloride inhibited shoot regeneration markedly at 100 and 150 mM NaCl. Both the fresh and dry weight were also reduced. The mineral contents (Na, K and Ca) of hypocotyl and cotyledonary cultures were disturbed at high levels of NaCl salinity. The osmotic potential (Ψs) was raised in hypocotyl and cotyledonary cultures in MS medium as the NaCl salinity level increased. Calcium enhanced shoot regeneration in hypocotyls and cotyledonary cultures, especially at the highest salinity level (150 mM NaCl). This calcium-induced counteraction of the harmful effect of NaCl may be due to the reduced uptake of Na and to the elevated water content of hypocotyls and cotyledonary cultures under Na-Ca combination.

Key words: tissue culture, tomato, salinity, calcium

Introduction

Soil salinity is a worldwide problem hampering the productivity of several agricultural crops. The utilization of the tissue culture technique to derive cell lines tolerant to NaCl stress is one approach to the improvement of salt tolerance in tomato (Dix and Street, 1975; Alfoceo et al., 1993). It has been reported that NaCl beyond 80 mM negatively affected the organogenesis of shoots and roots in tomato cell cultures (Tal et al., 1989). Calcium has long been used to overcome numerous types of stresses, which vary widely in effects and nature (Ruiz-Lozano and Azcon, 1997; Leopold and Willing, 1984; Cramer et al., 1988; Abdel-Basset and Issa, 1994; Hamada, 1996; Issa, 1996; Momonki et al., 1996). Calcium is often cited as a culture parameter affecting cell differentiation (Tanimoto and Ishioka, 1991; Roberts and Haigler, 1990). Increasing the calcium concentration of the culture medium increases the frequency of somatic embryogenesis in carrot (Janson et al., 1990; Monotoro et al., 1995; Etienne et al., 1997). Calcium acts both at the intracellular level (Poovaiah and Reddy, 1987) and at the apoplast level as an ion responsible for membrane stability and wall rigidity.

Calcium is considered as an important factor in the maintenance of membrane integrity and ion transport. It has also been reported that Ca is essential for K/Na selectivity. The mineral composition of plant tissues is affected by NaCl salinity. Nicolas et al. (1985) recorded increased K content in drought-stressed wheat plants. Such accumulated K may play a role in osmotic adjustment (Munns and Weir, 1981; Hamada, 1996). Elevated concentrations of Ca in the nutrient solution mitigated the adverse effect of NaCl to various

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extents by inhibiting Na uptake (Cramer et al., 1988) and reducing the leakiness of the membranes (Leopold and Willing, 1984).

The aim of the present work was to follow up the salinity-related impact on the regeneration capacity of tomato explants derived from either hypocotyls or cotyledons. In addition, the variability in salt tolerance of explants of different origins (hypocotyls and cotyledons) was investigated, as was the ameliorative effect of Ca^{2+} in overcoming salinity stress.

Materials and methods

Tomato (*Lycopersicon esculentum* Mill.) seeds were floated on distilled water for 15 min, surface-sterilized by immersion in 5% Clorex for 5 min, and then washed with distilled water three times. The seeds were germinated on Murashige and Skoog (1962) agar-solidified medium without hormones (MS-I). The hypocotyls and cotyledons were excised from 10-day-old seedlings grown *in vitro*. The excised explants were transferred in aseptic conditions to 50 ml conical flasks containing 20 ml of MS medium supplemented with (mg/l): 6 IAA, 5 kinetin, 40 adenine sulphate, 170 $NaH_2PO_4.H_2O$, 100 inositol, 0.1 thiamine-HCl, 0.5 pyridoxine, 0.5 nicotinic acid, sucrose (30 g l^{-1}) and agar (7.5 g l^{-1}) (MS-II). Sodium chloride was incorporated into the MS-II medium at different levels of NaCl (0, 25, 50, 100 and 150 mM) exerting osmotic potentials (O.P.) of –5.2 (control), –6.6, –9.1, –12.0 and –15.5 bars, respectively. Calcium (5 mM) was added as $CaCl_2$ in combination with NaCl in another series of subcultures. For all combinations, the pH was adjusted to 5.5±0.2 before autoclaving. Each flask contained 5 explants (hypocotyls or cotyledons), in three replicates. The cultures were incubated in an incubator at 27°C, continuously illuminated with fluorescent white light (376.8 µmol m^{-2} s^{-1}). After 21 days the fresh and dry weights were recorded.

Na and K determination

After the digestion of 20 mg dry mass in concentrated nitric acid for 1 h at 150°C till no further yellow vapour was produced (Kothari et al., 1990), Na and K were determined using the flame photometry method (Williams and Twine, 1960).

Osmotic potential

Ten mg dry tissue were extracted in boiling distilled water for 2 h. The filtrate was then used to estimate the osmolarity of the samples using an osmometer.

Statistical analysis

The data were analysed by one-way analysis of variance (PC-stat computer program) and least significant differences (LSD) were used to test the significance between treatments.

Results and discussion

In this work, tomato explants from cotyledons (cot) and hypocotyls (hyp) were grown for 21 days in culture media (MS-II) with or without 5 mM Ca^{2+}supplementation.

The efficiency of shoot organogenesis from hypocotyls and cotyledonary explants is shown in Table 1. The regeneration of shoots from cot explants was completely inhibited at the highest NaCl levels, while shoot regeneration from hyp explants was less affected. The addition of Ca to the culture medium raised the percentage of regenerated shoots in both hypocotyls or cotyledons especially at high salinization levels.

Table 1
Shoot regeneration of hypocotyl and cotyledonary explants, cultured for 3 weeks on MS-II supplemented with different levels of NaCl or NaCl + 5 mM $CaCl_2$

NaCl (mM)	Hypocotyl explants						Cotyledonary explants					
	No. of ERS		X of S/E		% of ERS		No. of ERS		X of S/E		% of ERS	
	0	Ca	0	Ca	0	Ca	0	Ca	0	Ca	0	Ca
0	27	35	1.22	2.5	100	130	9.0	12.0	6.0	9.5	100	130
25	27	30	1.22	3.0	100	100	7.0	9.0	4.7	6.7	100	100
50	15	24	0.88	2.1	55	88	6.0	8.0	4.0	7.6	65	80
100	12	21	0.55	2.0	44	77	–	6.0	–	5.8	–	65
150	–	18	–	1.5	–	65	–	5.0	–	4.5	–	50

S/E = Shoot/Explant; ERS= Explant regenerated shoots; X = average number of shoots per explant (S/E)

This result agrees with Komamine's (1988) model for carrot, in which a high concentration of $CaCl_2$ (200 mM) in a medium containing 2,4-D and zeatin is effective in increasing the number of cells able to develop to somatic embryos. Etienne et al. (1997) found that the addition of $CaCl_2$ into the culture medium of *Hevea brasiliensis* increased the regeneration capacity of somatic embryos. They added that the existence of an additional source of Ca^{2+} or the maintenance of Ca^{2+} gradients was essential for normal somatic embryo development. Eklund and Eliasson (1990) concluded that calcium in the nutrient solution stimulates cell wall deposition and cell wall synthesis, hence lack of Ca^{2+} in the nutrient medium makes them more sensitive to stress. In control cultures, cot explants exhibited a higher water content percentage than hyp explants (Fig. 1).

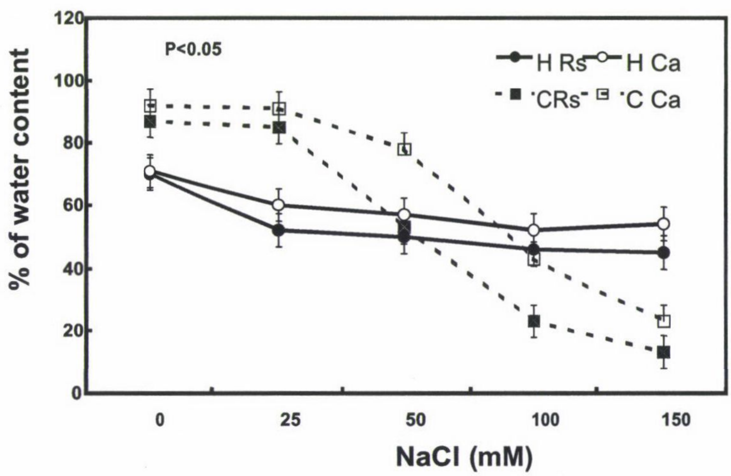

Fig. 1. Percentage water content of hypocotyl and cotyledonary explants cultured on MS-II medium supplemented with different levels of NaCl with or without calcium. H: hypocotyl, S: salinized without calcium, Ca: calcium supplemented, C: cotyledonary

Salinity lowered the water content in both explants, with a more severe effect on cotyledonary explants. In cot explants the water content dropped from 86.9 % to 12.5%, while in the hyp explants it only dropped from about 70.9 % to 45.7%. The severity of the salinity-induced drop in water content of the explants was most noticeable at the highest levels of NaCl (100 and 150 mM NaCl), where the percentage water content of the cot explants became lower than that of hyp explants. Ca^{2+} supplementation (5 mM) elevated the values of percentage water content in both hyp and cot explants, irrespective of the salinization level applied. Concomitantly, NaCl lowered the cellular osmotic potential, while it was elevated by Ca^{2+} relative to the corresponding reference culture containing only NaCl (Fig. 2). Cot explants were more responsive to Ca^{2+} supplementation regarding water content than hyp ones. Ca^{2+} induced maximum water content percentage at 100 mM NaCl (91.1%) followed by 150 mM (81.6%) in cot explant whereas a slight but continual decrease in osmotic potential by Ca^{2+} relative to NaCl salinity was exhibited in both of the explants. Elevated water content and/or decreased osmotic potential should constitute a better environment for the growth of explants under salinity stress conditions. Water exchange between explants and their environment *in vitro* clearly plays a major role in controlling the explant metabolism. A relationship between changes in the water status of the environment and the metabolism of callus has been clearly demonstrated in the organogenesis of rice callus (Lai and Liu, 1988) and tobacco cells (Brown et al., 1979; Hammersley-Straw and Thorpe, 1988). It was also found that low water content (rice) or very negative cellular water potential and osmotic potential (Ψs) in the callus appear to be good markers of embryogenic and organogenic phenomena.

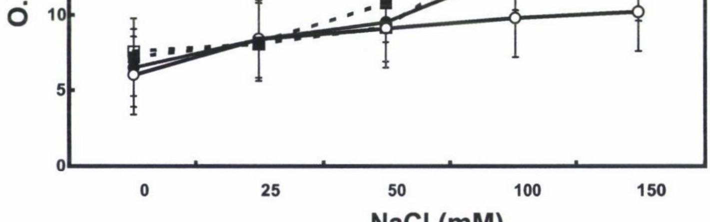

Fig. 2. Osmotic potential of hypocotyl and cotyledonary explants cultured on MS-II medium supplemented with different levels of NaCl with or without calcium. H: hypocotyl, S: salinized without calcium, Ca: calcium supplemented, C: cotyledonary

In somatic embryogenesis, it is often reported that reducing (Ψs) in the medium by adding permeating or non-permeating molecules stimulates the induction of embryos (Litz and Conover, 1983; Brown et al., 1989). Etienne et al. (1991) stated that the relative water content and water potential (Ψs) of the callus appear to be good markers of its embryogenic and organogenic state.

Tomato cot explants always exhibited higher fresh and dry weight than hyp explants. Increasing salinization levels lowered the weights of both hyp and cot explants (Figs 3, 4A and B). Although Ca^{2+}-supplemented cultures showed the same trend as salinized ones, in general, the protective effect of Ca^{2+} could be observed i.e. the inhibitory effect of NaCl existed but was slowed down by Ca^{2+}. In all cases, Ca^{2+}-containing cultures exhibited higher fresh and dry weights than those of the corresponding reference cultures containing only NaCl. Moreover, in combination with 25 mM NaCl, Ca^{2+} increased the fresh and dry weights of both hyp and cot explants compared with those of the control explants. The maxima of fresh and dry weight were observed to coincide with water content. In this respect, Banuls et al. (1997) recorded that calcium nitrate considerably increased the growth of *Citrus* plants exposed to chloride salts.

Na^+ was continually accumulated with the rise of the NaCl level in the growth media of both cot and hyp explants (Table 2). Cotyledonary explants contained higher levels of Na^+ than hyp explants, which would be expected, due to the known toxicity of Na^+, to retard the growth of cot in comparison with hyp explants. However, this was not the case, indicating that cot explants are more salt-tolerant than hyp explants. Na^+ content is not growth limiting in this case. Supplemental Ca markedly lowered the rate of Na^+ accumulation, most noticeably at the highest concentrations of NaCl. Contrary to Na^+, the K^+ contents of salt-stressed explants were significantly lowered by salinity. The higher the level of imposed salinity, the lower the K^+ content observed, indicating the leakiness of the membranes. Generally, salt-stressed cells are known to be leaky due to loss of membrane integrity.

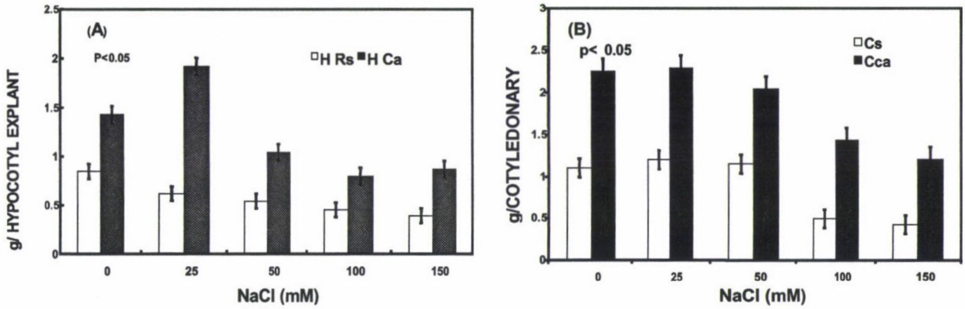

Fig. 3. Fresh weight of hypocotyl- (A) and cotyledonary (B) explants cultured on MS-II medium supplemented with different levels of NaCl with or without calcium. H: hypocotyl, S: salinized without calcium, Ca: calcium supplemented, C: cotyledonary

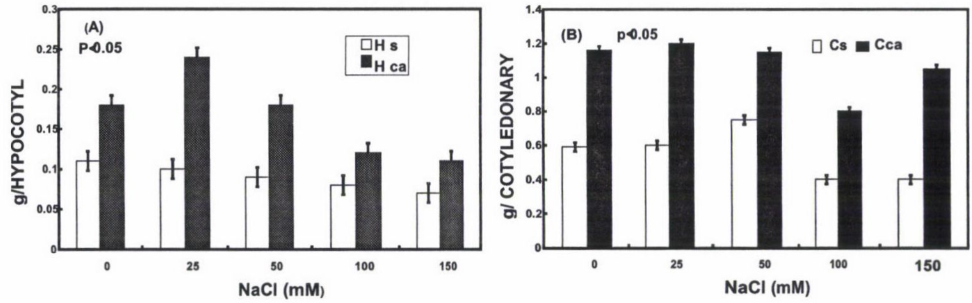

Fig. 4. Dry weight of hypocotyl (A) and cotyledonary (B) explants cultured on MS-II medium supplemented with different levels of NaCl with or without calcium. H: hypocotyl, S: salinized without calcium, Ca: calcium supplemented, C: cotyledonary

One of the accepted criteria of injured membranes is K^+ leakage, which is ameliorated by Ca^{2+}. Thus, Ca^{2+} maintained K^+ contents relatively higher than those of the corresponding reference cultures containing NaCl without supplemental Ca^{2+}. Membranes tend to be more leaky under salt stress conditions and it has been shown that Na^+ displaces Ca^{2+} from the membranes (Cramer et al., 1988). Supplemental Ca^{2+} stabilizes the membranes and leads to reduced permeability. More obvious than the K^+ contents are the K^+/Na^+ ratios, which dropped sharply upon the addition of NaCl even at its lowest level (25 mM). Ca^{2+} addition prevented this effect of salinity.

Thus, elevated values of percentage water content, K^+ content and K^+/Na^+ ratios, together with lower osmotic potential and decreased Na^+ content are growth-stimulating factors. Since supplemental Ca^{2+} improved these criteria, the ameliorative role of Ca^{2+} against salinity stress is thus confirmed.

Table 2

Na^+, K and K/Na ratio of hypocotyl and cotyledonary explants cultured on MS-II medium with different levels of NaCl with or without 5 mM $CaCl_2$

NaCl (mM)	Hypocotyl explants						Cotyledonary explants					
	0			Ca			0			Ca		
	K	Na	K/Na	K	Na	K/Na	K	Na	K/Na	K	Na	K/Na
0	54.5	4.8	11.4	36.1	5.2	6.9	62.4	9.6	7.7	54.7	7.1	7.7
25	36.2	7.9	4.9	35.6	3.2	11.3	54.9	9.1	7.2	52.1	12.6	4.1
50	30.8	7.3	4.2	31.4	6.9	4.7	46.9	15.3	3.1	55.6	14.2	3.9
100	34.1	12.9	2.8	35.3	8.7	4.1	52.2	19.1	2.7	34.5	14.8	2.3
150	36.7	16.4	2.1	45.2	11.0	4.1	31.7	21.4	1.4	32.2	12.4	2.6
$LSD_{5\%}$	5.15	2.23	0.76	7.3	1.73	1.94	6.42	1.38	1.83	4.5	1.16	0.33

Significant difference ($P < 0.05$).

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NO_3^- AFFECTS CARBOHYDRATE LOSSES FROM WHEAT ROOTS

M. BENDRISS AMRAOUI and AHMED TALOUIZTE

DEPARTMENT OF BIOLOGY, FACULTY OF SCIENCES DHAR EL MEHRAZ, ATLAS, FES, MOROCCO

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The influence of NO_3^- on carbohydrate (C) losses from the roots of 21-day-old wheat seedlings was studied under light and N supplies ranging from deficient to excessive (0–8 mM NO_3^-). C loss is not influenced by the quantity of internal soluble carbohydrates (SC), but is affected by the nitrate status of the seedlings. In low illuminance, the NO_3^- loss is significantly higher than that in high illuminance, whereas C loss is significantly higher in high illuminance than in low illuminance, in spite of there being little difference between the seedlings of both light treatments in the SC concentration in the roots at 0 h, suggesting the existence of a negative correlation between NO_3^- and C efflux and a close relationship between the C efflux system and NO_3^- assimilation. Low NO_3^- and light reduced the C loss, which was decreased to a greater extent by low NO_3^- than by low light, indicating that C loss was more dependent on NO_3^- than on C export. The high decline in C loss, irrespective of whether there is an increase in NO_3^- loss (i.e. in low light) or a decrease in NO_3^- loss (i.e. at low nitrate), may indicate that the two types of losses involve different mechanisms.

Key words: C efflux, NO_3^- efflux, nitrate status, C accumulation, N starvation

Introduction

It is well established that the carbon and nitrogen metabolisms are linked, because they must share organic carbon and energy supplied directly from photosynthesis or from the respiration of fixed carbon (Talouizte et al., 1984a; Van Quy et al., 1991; Van Quy and Champigny, 1992; Huppe and Turpin, 1994; Champigny, 1995; Améziane et al., 1997). It has been found that the capacity of wheat roots to assimilate nitrogen is correlated directly with their C status, and the carbon skeletons for nitrogen assimilation derive from the stored carbon (Champigny and Talouizte, 1986). There is increasing evidence that NO_3^- considerably influences the source-sink relations and sucrose metabolism in roots (Pollock and Cairns, 1991; Améziane et al., 1997). Most research on nitrogen effects has been performed on anaplerotic CO_2 fixation and sucrose synthesis. It is known, for example, that PEPcase (EC 4.1.1.31) is activated and SPS (EC 2.4.1.14) activity is decreased (Van Quy et al., 1991; Champigny, 1995), but very little is known about the integration of the C catabolism into the nitrogen metabolism and its regulation by N in sink tissues (Stitt and Steup, 1985; Pollock and Cairns, 1991; Huppe and Turpin, 1994; Champigny, 1995). Little is also known about the destiny of the hexose formed either during fructan synthesis (Pollock and Cairns, 1991) or during sucrose, fructan and starch degradation. It is believed that sucrose synthesis occurs from free hexose in sink tissues as well as in source leaves because of the relatively low tissue contents (Wagner et al., 1983; Lucas and Madore, 1988), but the magnitude of such recycling and the effect of nitrogen on this process have not been well understood. In tubers of *H. tuberosus,* Pollock and Chatterton (1988) found that chilling induced a net depolymerization of fructan with no net C loss, indicating resynthesis of short-chain fructan acceptors. It has also been found that N deprivation increases sucrose-sucrose fructosyl transferase (SST, EC 2.4.1.99) activity and probably fructan synthesis (Améziane et al., 1997), but to our knowledge little is known about the recycling and loss of C during fructan synthesis in N-limited conditions or about the possible relationships between the NO_3^- metabolism and C loss in wheat in response to N enrichment. Since NO_3^- may affect the export and storage of C and tissue sink strength, it may eventually affect, in accordance with its concentration in the middle and light intensity, the composition, patterns and rates of C release from the roots, the effects of which are well known to be a basic character of mycorrhizal symbiosis (Martin et al., 1987), but whose physiological significance and mechanism are not well known (Bush, 1993). The hypothesis that NO_3^- supply will affect the destiny of C in wheat roots is tested by determining the concomitant C and NO_3^- losses from (1) seedlings grown at three levels of NO_3^- supplies ranging from deficient to luxurious at high light level and from (2) seedlings grown on NO_3^- at low light level. The aim of the present paper was to examine the effect of NO_3^- and light intensity on the C efflux from roots.

Materials and methods

Plant material and growing conditions

Wheat seeds (*Triticum aestivum* L., var. Saba) disinfected for 5 min in sodium hypochlorite (37 g/l) were soaked for 8 h in distilled water and then allowed to germinate for 7 days on moist cheesecloth in darkness at 18 to 20°C. Seedlings were planted in groups of 25 in plastic holders and placed in 5 litres NO_3^- solution. The NO_3^- solution at pH 5.5 contained 3 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 0.75 mM KH_2PO_4, 0.25 mM K_2HPO_4, 0.2 mM NaCl, 0.75 mM $MgSO_4$, 10 µM $MnCl_2$, 6 µM $ZnSO_4$, 48.5 µM H_3BO_4, 16.5 µM Na_2MoO_4 and 0.5 µM $CuSO_4$. Iron was added as 10 mg of Fe^{3+}-EDTA per litre of NO_3^- solution. The solutions were constantly aerated and replenished every three days. Growth was carried out in a controlled chamber where light was applied during a 16 h photoperiod by white fluorescent bulbs (mixolamp 160W) and tubes (40W). The temperature was 21°C in the light period and 18°C in the dark period. Relative humidity was about 65%.

Nitrogen and light treatments

In the high N treatment (+N), seedlings were constantly grown on NO_3^- solution for 14 days at low illuminance (LI) 2000 or high illuminance (HI) 4000 lux at pot level. In the 10d(–N) treatment, 4 days after planting out into NO_3^- nutrient solution at 4000 lux, seedlings were transferred for 10 days at 4000 lux onto N-free nutrient solution similar to the complete solution except that 3 mM KCl and 2.5 mM $CaSO_4$ were used to replace the NO_3^- salts. In the 21d(–N) treatment, after 7 days of germination, seedlings were constantly grown on N-free nutrient solution for 14 days at 4000 lux.

Plant treatments

On the 21st day after soaking, 3 h after the start of the light period, four intact plant roots were rinsed three times with distilled water and placed under the same illuminance used for growth in a test tube containing 40 ml of distilled water, at pH 5.5. The solution was constantly aerated. At the end of the first sampling period, the roots were placed in another 40 ml of distilled water for another period. The sequence was continued through 8 h and the medium was changed after each period. Seedlings were sampled at the beginning and end of the experimental period (8 h); roots and shoots were excised, dried and weighed. Tissue nitrate and SC before and after transfer of seedlings (at 0 h) to bathing solution were extracted from root and shoot separately with a cold methanol: chloroform: H_2O (12: 5: 3 v/v/v) mixture according to Talouizte et al. (1984b). Exudates from the roots to water at different times were concentrated separately in 2 ml of distilled water. The NO_3^-–N content of tissues and root exudates were determined with the method of Cataldo et al. (1975). SC of tissues and C of root exudates were assayed by the anthrone method (Halhoul and Kleinberg, 1972) and expressed as glucose equivalent. In the figures, SC and C loss are given as μg glucose per root dry weight and NO_3^- as μg NO_3^-–N per root dry weight.

Data analysis

The data were analysed by ANOVA (analysis of variance) to prove the significant difference between the treatments. Standard errors were calculated for all means and where no error bars appear the standard error falls within the size of the symbol. For each treatment four repetitions (each of four plants) were made. For each repetition root exudates were collected during 0–8 h from the same plants. Each point of the graph represents a single sampling time and is the mean of four repetitions with measurements made on different plants.

Results and discussion

Roots must use different strategies than do photosynthetic tissues to support N assimilation because translocated photosynthates provide both carbon skeletons and all the energy to roots and hence regulation is required to prevent potential competition between NO_3^- assimilation and other metabolic pathways (for example: NO_3^-/sucrose synthesis). The assimilation of NO_3^- by the roots may require the regulation of the synthesis, translocation and supply of photosynthates, together with organic acids, to the mitochondria (Weger and Turpin, 1989; Bloom et al., 1991; Huppe and Turpin, 1994; Améziane, 1997). In the roots, photosynthates may be degraded, stored in the vacuole or leaked out (Wagner et al., 1983; Martin et al., 1987; Pollock and Cairns, 1991; Bush, 1993). Roots are known to continuously exude several compounds, i.e. organic acids, carbohydrates and amino acids (Martin et al., 1987). The composition and the physiological significance of root exudates have been extensively investigated in tree mycorrhizae studies (Martin et al., 1987), but less research has been done on cereals (Gransee and Wittenmayer, 2000) and on the regulation and integration of this process in the root nitrogen metabolism.

Nitrogen deficiency increases the accumulation of SC (Fig. 1). This may be due to low SC degradation related to low NO_3^- assimilation and synthesis of amino acids (Rufty et al., 1988; Weger and Turpin, 1989; Bloom et al., 1991). It may also be due to (i) the recycling of C formed either during C degradation or during fructan synthesis, which increases under N-limited conditions (Améziane et al., 1997), (ii) the use of organic acids as respiratory substrates instead of C, (iii) the loss of respiratory enzymes or mitochondria and (iv) a decrease in the C permeability of root membranes. The root SC does not increase in 10d(–N) seedlings, whereas it significantly increases when the seedlings have never received NO_3^- (i.e. in 21d(–N) seedlings).

In contrast to this, the shoot SC significantly increases in all (–N) seedlings, indicating that the shoot carbon metabolism was probably more affected by N starvation than that of the roots (Fig. 1). This result may indicate that in 10d(–N) seedlings, probably neither excess C export from the shoots to the roots nor a decline in respiratory C degradation in the roots occurred during N starvation. However, during the 8–h experiment, the shoots accumulated 88, 95.6 and 39% of initial shoot SC (at 0 h), whereas the roots accumulated 34, 87 and 36% of initial root SC (at 0 h) in (+N), 10d(–N) and 21d(–N) seedlings, respectively. This shows that even if the roots of 10d(–N) seedlings accumulated 87% of the initial root SC they could not store it in their vacuoles because at 0 h the low root SC contents found in these seedlings were similar to that of (+N) seedlings at the same time (0 h).

A high proportion of the C quantity (87%) translocated to the roots in these seedlings will probably be consumed by respiration in the later dark period, suggesting fewer changes in their root respiration during starvation. It may also show that in 21d(–N) seedlings, even if photosynthesis was decreased by N starvation, the shoots were able to maintain C export to the roots. On the other hand, if we compare the SC of (–N) seedlings to that of (+N) seedlings, we find that even if 21d(–N) seedlings appear to accumulate more SC during N starvation, they do not export more C to the roots than (+N) and 10d(–N) seedlings during 8 h, probably indicating greater alterations in their root respiration caused by starvation (Fig. 1).

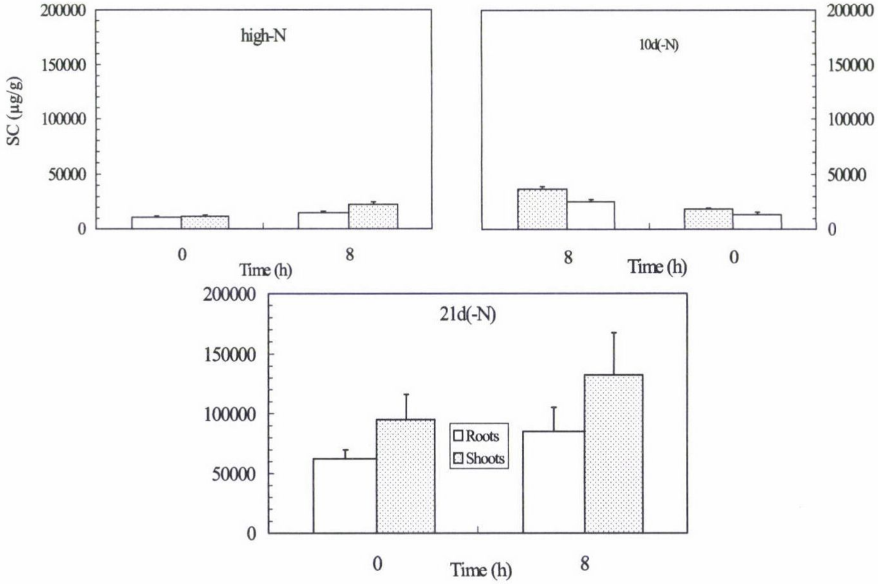

Fig. 1. Soluble carbohydrate concentrations (SC) in roots and shoots before (0 h) and after (8 h) transfer of intact roots of 21-day-old wheat seedlings to bathing solution. Values are the mean ± SE of four repetitions

This result may indicate that the increase in root SC during starvation may stem from an increase in C exports and changes in respiration, but probably also from other processes. However, although it has been observed that the carbon export (efflux) from source leaves depends on their sucrose concentration (Ho, 1988), the present data show that here C loss from the roots is little influenced, if at all, by the size of the internal SC content, but is more dependent on the nitrogen status of the seedlings (Fig. 2A). (+N) seedlings, which showed low C accumulation and also low C export into roots during 8 h, lost a higher quantity of C compared to (–N) seedlings, indicating that C loss is very sensitive to N availability (Figs 1 and 2A).

In contrast to this, it has been reported that the total amount of carbon recovered in the root exudates decreases with increasing N fertilization (Kuzyakov and Domanski, 2000) and is controlled by the availability of N and carbon substrates in the roots (Lemaire and Millard, 1999). It has also been reported that the carbon-replete plants whose growth is N-limited have an increased allocation of carbon to roots and a greater rate of exudation of carbon substrates compared to plants grown with a carbon-limitation, suggesting that here (–N) seedlings have probably leaked other carbon compounds instead of carbohydrate. More detailed analysis is needed to investigate this possibility. However, the high decline in C loss from (–N) seedlings, which ranged from 86 to 91% of the C loss from (+N) seedlings, indicates that the increase in root SC in the case of N starvation may also be due to changes in C loss. These may not be entirely due to a decline in respiration: since N starvation induces an increase in root intra-cellular C content and root growth, the respiration may not be drastically decreased, because it must provide the energy and carbon skeletons required for root growth. Furthermore, the fact that the roots of 10d(–N) seedlings lost little C during 8 h (Fig. 2A) even if their SC content was similar to that of (+N) seedlings, probably due to fewer changes in root respiration, suggests that the lower C loss from the roots may also be due to the inactivation or degradation of the membrane protein responsible for the C efflux caused by N starvation, as reported for NO_3^- efflux by Van der Leij et al. (1998).

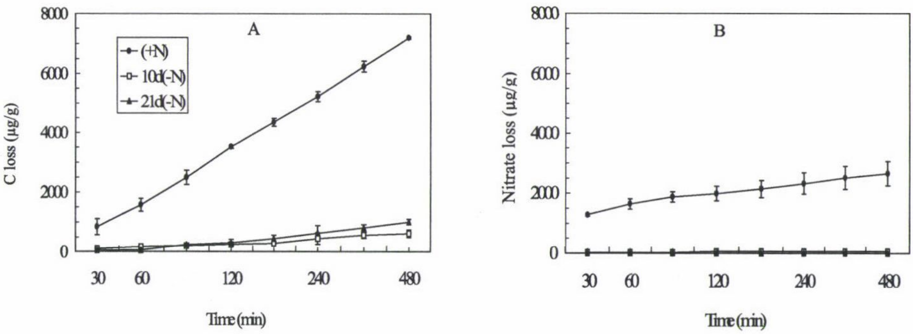

Fig. 2. Effect of N deprivation on the time course of cumulative carbohydrate (A) and nitrate (B) losses from intact roots of 21-day-old wheat seedlings following transfer (at 0 h) to bathing solution as described in the Materials and methods section. Values are the mean ± SE of four repetitions

In contrast to this, the high C loss from (+N) seedlings was probably due to the maintenance of high C loss system activity induced by a concomitant increase in sucrose degradation and a decrease in fructan synthesis and C recycling caused by the nitrate-induced decrease in SPS and SST activities in the roots as found for SPS in leaves (Van Quy and Champigny, 1992; Champigny, 1995). The stimulation of C loss by NO_3^- supply and the decline caused by N starvation confirm the hypothesis that the rate of biosynthesis (processes utilizing ATP: nitrate assimilation), rather than C, is the main limiting factor for the supply of C to the mitochondria and for respiration (Journet et al., 1986) and probably also for the synthesis and activity of the C efflux system.

On the other hand, an increase in the respiratory oxidation of C and the condensation of simple C into polysaccharides were shown in (–N) wheat seedlings (Talouizte et al., 1984a). Additionally, an increase in the malate concentration was also observed in the vicinity of the root tips in response to N starvation (Stumpf and Burris, 1981). However, in (+N) seedlings few organic acids (OA) are likely to be stored, because they may be used to buffer the OH^- generated during nitrate reduction and to replace the NO_3^- anion for charge balancing.

Recently, Gransee and Wittenmayer (2000) found that the proportions of C and OA in root exudates vary with plant species and growth stage. They also found that during plant development the relative amount of C decreased at the expense of OA, suggesting that the C efflux system was very active at very early growth stages. These observations suggest that here in (–N) seedlings, OA may replace C as the respiratory substrate, leading to a decline in C loss and an increase in OA loss. They may also suggest that the change in the kind of respiratory substrate and the extent to which this replacement occurs in relation to N supply are probably linked to the synthesis and activity of the C efflux system.

However, if here it is assumed that in (–N) seedlings OA are the main respiratory substrates, the C loss from these seedlings is likely to be due to fructan synthesis and C recycling or to the low respiration rate if the C efflux channel remains active. Taken together, these results suggest that in the presence of NO_3^-, either the decrease in root fructan and OA synthesis or the increase in respiration, sucrose degradation and the C efflux system activity in roots, singly and/or in interaction, may increase C loss, but the contribution of each of these factors to variations in C loss cannot be determined here.

Since C loss was affected by the NO_3^- supply and probably by membrane permeability, is it linked to NO_3^- efflux? To reply to this question, the variation in NO_3^- efflux in root exudates was also investigated. However, when C and NO_3^- releases are compared, it is found that NO_3^- loss, as for C loss, follows internal nitrate contents and is thus relatively important for (+N) seedlings but not at all so for 10 and 21d(–N) seedlings (Figs 2A, 2B and 3). Loss of NO_3^- from (+N) seedlings increased slowly and remained lower than that of C, whereas for all (–N) seedlings losses of C and NO_3^- were very low, suggesting the existence of a relationship between losses of NO_3^- and C. What, then, happens to C loss if NO_3^- loss increases? Is it increased by NO_3^- loss?

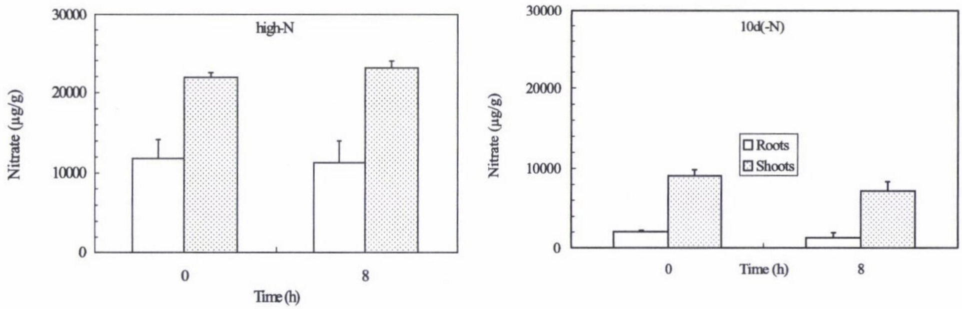

Fig. 3. Nitrate concentrations in roots and shoots before (0 h) and after (8 h) transfer of intact roots of 21-day-old wheat seedlings (+N and –N) to bathing solution. Values are the mean ± SE of four repetitions

To test the hypothesis that C and NO_3^- losses are linked, they were compared under two light intensities (low, LI, and high HI, illuminance) as described in the Materials and methods section. The effect of low NO_3^- assimilation (i.e. increased NO_3^- loss) was also tested here without altering the NO_3^- supply or, probably, the membrane structure and permeability. However, during 0–8 h, seedlings exposed to HI accumulated relatively high amounts of SC, whereas at LI, seedlings did not accumulate SC (Fig. 4), indicating low SC formation and hence low translocation from the shoots to the root in LI, leading to low NO_3^- assimilation and thus to a high accumulation of NO_3^- in the tissues (Fig. 5).

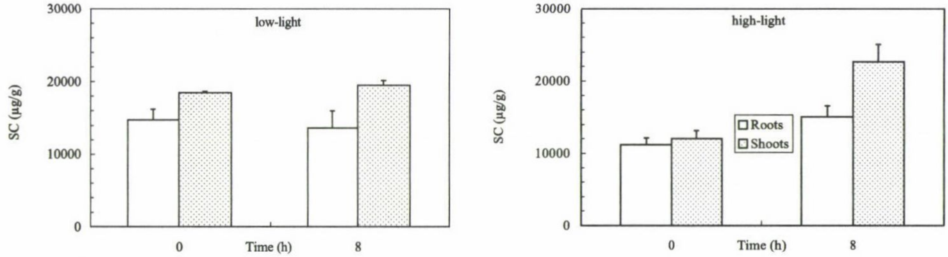

Fig. 4. Effect of light intensities on the soluble carbohydrates in the roots and shoots of 21-day-old wheat seedlings before (0 h) and after (8 h) transfer to bathing solution. Values are the mean ± SE of four repetitions

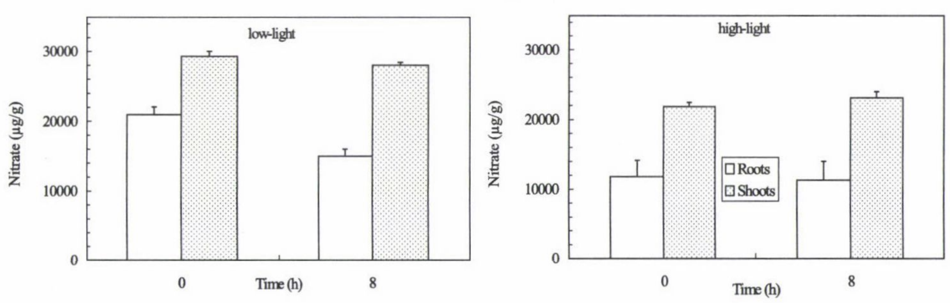

Fig. 5. Effect of light intensities on the nitrate contents in the roots and shoots of 21-day-old wheat seedlings before (0 h) and after (8 h) transfer to bathing solution. Values are the mean ± SE of four repetitions

The question is, whether this increase in root NO_3^- content induces a concomitant increase in C and NO_3^- losses in spite of the decline in NO_3^- assimilation, and from what compartment the effluxed C comes. NO_3^- loss from LI seedlings was significantly higher than that from HI seedlings, whereas C loss was significantly higher at HI than at LI, in spite of there being little difference between HI and LI seedlings in root SC concentration at 0 h, suggesting a negative correlation between NO_3^- and C efflux (Figs 4 and 6).

Robinson and Baysdorfer (1985), Weger and Turpin (1989) and Bloom et al. (1991) have reported a close relationship between NO_3^- efflux and light, between NO_3^- efflux and C and between NO_3^- efflux and respiration via nitrate assimilation. It is well known that a reduction in light intensity may cause a substantial decrease in photosynthesis and C translocation, leading to the depletion of the intra-cellular C content and a subsequent decrease in respiration (Saglio and Pradet, 1980). The high increase in C loss associated with a high decline in NO_3^- loss at HI, in spite of there being little difference between the root SC contents of HI and LI seedlings at 0 h, suggests that at LI it is the low C export which affects C loss by decreasing the NO_3^- assimilation through a diminution of the current photosynthate degradation and carbon supply to the mitochondria. This may eventually also decrease C loss by closing the C efflux channel and/or by stimulating hexose recycling during the use of reserves by respiration. However, the degree to which current photosynthates or stored assimilates can be used in this process will require much more research with ^{14}C tracers to determine the distribution of current photosynthates between C loss and storage pools in the roots.

Finally, the present data indicate that C loss was dependent on both light and NO_3^- pre-treatment via nitrate assimilation (Figs 2 and 6). Low NO_3^- and light reduced C loss. C loss was decreased to a greater extent by low NO_3^- than by low light, indicating that C loss was more dependent on nitrate than on C export. The high decline in C loss in spite of an increase or decrease in NO_3^- loss, reported in this paper, may indicate that the two types of losses involve different mechanisms. Further studies on respiration, fructan synthesis (including hexose recycling) and organic acid synthesis in the roots in relation to the nitrate supply may contribute to the elucidation of these assumptions and to a better understanding of the mechanism of the carbohydrate efflux system.

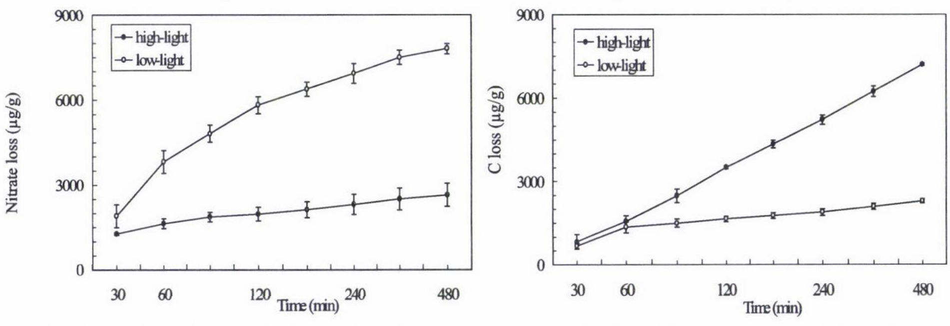

Fig. 6. Effect of light intensities on the time course of cumulative nitrate and carbohydrate losses from intact roots of 21-day-old wheat seedlings following transfer (at 0 H) to bathing solution. Values are the mean ± SE of four repetitions. Differences between N treatments were tested by ANOVA (analysis of variance, $P < 0.05$)

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ROLE OF DIFFERENT GENOME COMBINATIONS ON STABILITY PARAMETERS IN WHEAT AND TRITICALE

S. ARUMUGAM and V. R. K. REDDY

CYTOGENETICS LABORATORY, DEPARTMENT OF BOTANY, BHARATHIAR UNIVERSITY, COIMBATORE, TAMIL NADU, INDIA

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The role of the different genome combinations in a polyploid on phenotypic stability was analysed in wheat and triticale. Twelve genotypes with four genome combinations (AABB, AABBDD, AABBRR and AABBDDRR) were raised in eight artificially created environments. The data on grains per spike, 100-grain weight and grain yield per plant were recorded and analysed following the models of Perkins and Jinks (1968) and Eberhart and Russell (1966). The results revealed that in polyploid species the genes for stability were not uniformly distributed in different genomes. It was therefore inferred that stability may largely depend on the gene combination rather than on the genome combination.

Key words: polyploid, genome, stability

Introduction

Genotypes possessing high yield potential coupled with stable performance in different environments have great value in plant breeding. The potential of genotypes and the stability of their performance can be judged by multi-environment testing. A breeding programme aimed at developing stable varieties needs information on the extent of genotype × environment interactions for yield and associated traits. Studies revealed that stability is under genetic control. No studies were made earlier to find out the role of the different genomes in a polyploid on phenotypic stability. Triticale, which is a polyploid genotype having A, B, R and D genome chromosomes, provides an ideal material for this study.

Materials and methods

Twelve genotypes belonging to four genomic combinations were selected for the present study. The four genome combinations were (i) Tetraploid wheat (AABB), (ii) Hexaploid wheat (AABBDD), (iii) Hexaploid triticale (AABBRR) and (iv) Octoploid triticale (AABBDDRR). Three genotypes or lines were taken for each genome combination (Table 1). Eight micro-environments were created using combinations of two fertilizer regimes, two sowing dates and two spacings as per the details given in Table 2. The experiment was carried out in a randomised block design consisting of three replications in each environment. Three rows of each genotype were raised in each environment and each replication. Data on grains per spike, 100-grain weight (g) and grain yield (g) per plant were recorded from five plants selected from the middle row of each generation. The recorded data were analysed following Perkins and Jinks (1968) and Eberhart and Russell (1966).

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Table 1
Details of the wheat (4x, 6x) and triticale (6x, 8x) genotypes used in the stability analysis

Sl. No.	Genotype	Genome combination	Variety/line
1	Tetraploid wheat	AABB	PBW 216 RAJ 911 HI 8381
2	Hexaploid wheat	AABBDD	HUW 318 NILGIRI SONALIKA
3	Hexaploid triticale	AABBRR	TL 419 JNIT-170 UPT-78268
4	Octoploid triticale	AABBDDRR	8A 94 8A 195 TCLUM OCTO

Table 2
List of environments created for the stability analysis

Manure status	Sowing dates			
	5th June 1996		25th June 1996	
	spacing		spacing	
Fertilised*	15 × 30 cm^2	15 ×15 cm^2	15 × 30 cm^2	15 ×15 cm^2
Unfertilised	15 × 30 cm^2	15 × 15 cm^2	15 × 30 cm^2	15 × 15 cm^2

*N =120 kg/ha (60+60); K = 60 kg/ha; P = 60 kg/ha (values were obtained using urea - 46% N, muriate of potash - 40% K and double super-phosphate - 32% P).

Results and discussion

The joint regression analyses designed by Perkins and Jinks (1968) revealed that the genotypes and the mean squares for environment were highly significant for all three characters, namely grains per spike, 100-grain weight and grain yield per plant (Table 3). This indicated the presence of significant variations due to environments. The significant G×E mean squares for the three characters showed the interaction of the genotypes with the environment. The G×E had two components, linear and non-linear (remainder). The mean squares of the linear component, when tested against error mean squares, were found to be significant for all the three characters. The mean squares of the non-linear component were found to be significant for grains/spike and 100-grain weight. In cases where both linear and non-linear components were found to be significant, the mean squares due to the linear component were tested against the mean squares due to the non-linear components. The mean squares for the linear component of grains per spike showed significance against the non-linear (remainder) mean squares. This indicated that despite the significant non-linear component in the G×E interaction, the linear component was predominant for this character (grains per spike). The 100-grain weight showed significant variation for both the linear and non-linear components when tested against the error mean squares, indicating that the G×E interaction was shared both by the predictable and unpredictable components. Since it showed a non-significant linear component when tested with the corresponding non-linear (remainder) mean squares, the linear and non-linear components contributed equally to the total G×E interaction. Grain yield/plant showed a significant linear component and a non-significant non-linear component indicating that this character was governed by predictable components.

Stability parameters such as mean (X), regression coefficient (b) and deviation from regression (S^2d) were also calculated following Eberhart and Russell (1966) in twelve genotypes for three characters (Table 4). According to Singh (1983) the model proposed by Eberhart and Russell (1966) is a simple and effective method for stability analysis. The effect of different genome combinations on stability can be revealed using the observed results.

Table 3

Joint regression analysis for three characters of twelve wheat and triticale genotypes grown over eight environments

Source of variation	Df	Grains/spike	100-grain weight	Grain yield/plant
Genotype	11	1176.2138**$^{xx++}$	3.9864**$^{xx++}$	104.3937**$^{xx++}$
Environment	7	93.1168**$^{xx++}$	1.733**$^{xx++}$	1.0070**$^{xx++}$
Gen. × Env.	77	12.7408^{xx}	0.1176^{xx}	0.1131^{xx}
Linear	11	22.9148^{xx+}	0.1785^{xx}	0.2394^{xx}
Non-linear (remainder)	66	11.0464^{x}	0.1082^{xx}	0.0920
Pooled error	176	7.8621	0.0307	0.0149

*, **: Significant at 5% and 1% levels of probability, respectively, when tested against Genotype × Environment mean squares; x, xx: Significant at 5% and 1% levels of probability, respectively, when tested against error mean squares; +, ++: Significant at 5% and 1% levels of probability, respectively, when tested against remainder mean squares.

Table 4

Estimates of stability parameters: mean (X), regression coefficient (b) and deviation from regression (S^2d)

Genotypes	Grains/spike			100-grain weight			Grain yield/plant		
	X	b	S^2d	X	b	S^2d	X	b	S^2d
4x wheat									
PDW 216	45.24	1.49**	0.37	4.50	0.68*	0.04**	12.94	0.82*	0.09
RAJ 911	43.53	1.06*	0.08	3.99	1.40**	0.33**	13.61	0.85*	0.07
HI 8381	40.13	0.19^{++}	–0.69	3.60	0.57^{+}	0.28	15.76	0.90*	0.12
6x wheat									
HUW 318	49.52	0.72	21.62**	5.32	0.57^{+}	0.02	17.49	1.16*	0.12
NILGIRI	45.66	1.39**	0.70	4.29	0.91*	0.09**	15.69	0.85*	0.10
SONALIKA	40.93	1.44**	0.33	4.41	1.20**	0.00	16.74	1.17*	0.17**
6x triticale									
TL 419	58.32	1.84**	13.58**	3.52	1.41**$^{+}$	0.00	14.65	0.98*	0.11
JNIT-170	62.32	1.82**	13.71**	3.95	0.97**	0.07**	12.81	0.94*	0.14*
UPT-78268	56.35	2.00**	9.09**	4.46	1.19**	0.23**	13.55	1.12*	0.09
8x triticale									
8A 94	23.93	0.63	0.31	2.87	0.98	0.06**	7.68	0.94*	0.15*
8A 195	26.13	0.64	0.04	4.87	1.14**	0.00	13.75	1.14**	0.11
TCLUM OCTO	32.01	0.48	0.85*	3.36	1.01**	0.13**	14.47	1.03*	0.11

*, ** - Significant at 5% and 1% levels of P, respectively; +, ++:'b' deviates significantly from unity at 5% and 1% levels of P, respectively.

Tetraploid wheat (AABB)

Two (PDW 216 and RAJ 911) of the three genotypes of tetraploid wheat had significant regression mean squares. Therefore these two had average responsiveness with respect to the character grain yield per spike. HI 8381 showed a significant variation of the regression mean square from unity, which revealed that it had no interaction with the environment. None of the three genotypes showed a significant deviation (S^2d) from regression. Since the varieties PDW 216 and RAJ 911 had significant regression mean squares and showed non-significant variation from regression, they were stable for this character. HI 8381 had a regression mean square deviating significantly from unity for 100-grain weight, indicating that it had no interaction with the environment. The other two genotypes, PDW 216 and RAJ 911, had significant regression mean squares and also had significant variation from regression, denoting that any predictions made for the performance of these genotypes in any environment would have limited reliability. Therefore, none of the tetraploid wheats could be classified as stable for 100-grain weight. With regard to grain yield per plant all three genotypes showed significant regression mean squares. They did not deviate significantly from unity. None of the genotypes showed a significant deviation from regression. Therefore, all three varieties (PDW 216, RAJ 911 and HI 8381) were found to be stable for this character. If the AABB genome combination is considered to be stable for characters where fifty per cent or more of the genotypes in the group were stable, it was found to be stable for the characters number of grains per spike and grain yield per plant, but unstable for 100-grain weight.

Hexaploid wheat (AABBDD)

Two of the three genotypes (Nilgiri and Sonalika) had significant regression mean squares, which did not deviate significantly from unity. Neither of these genotypes had a significant variation from regression. Therefore, these two genotypes (Nilgiri and Sonalika) were classified as stable for grains per spike. For the character 100-grain weight, the two genotypes (Nilgiri and Sonalika) showed significant regression mean squares variation, though Sonalika showed no significant variation from regression. Only this genotype could be classified as stable for 100-grain weight. Except Sonalika, the other two varieties (HUW 318 and Nilgiri) were classified as stable for grain yield per plant, since they had significant regression mean squares which did not deviate from unity. No significant variation from regression was noted in these two varieties. Considering the characters for which more than fifty per cent of the genotypes of this group were stable, it was found that the hexaploid wheats were stable for two characters, namely, grains per spike and grain yield per plant, and were unstable for 100-grain weight.

Hexaploid triticale (AABBRR)

All three genotypes had significant regression mean squares and significant deviation from regression for grains per spike. Therefore, none of the genotypes could be classified as stable. None of the three hexaploid triticales were stable for the character 100-grain weight, because two of them showed significant deviation from regression and the other had a regression mean square which deviated significantly from unity. All three genotypes showed significant regression mean squares and did not deviate from unity for grain yield per plant. However, JNIT-170 showed significant variation (S^2d) from regression. Therefore, TL 419 and UPT 78268 were found to be stable for this character. Considering the AABBRR genome combination to be stable for characters where fifty per cent of the genotypes belonging to the group were stable, it was found that this genome combination was only stable for grain yield per plant.

Octoploid triticale (AABBDDRR)

None of the octoploid triticales were stable for grains per spike, because none of the genotypes had significant regression mean squares. The genotype 8A 195 alone was considered as a stable genotype for 100-grain weight, since it had a significant regression mean square which did not deviate from unity and a non-significant variation (S^2d) from regression. With regard to grain yield per plant all three genotypes had significant regression mean squares, while one (8A 94) showed significant variation (S^2d) from regression. Therefore, the other two, namely 8A 195 and TCLUM OCTO, were found to be stable for this character. A comparative study of the stability of different genome combinations for the characters is given in Table 5.

The AABBDDRR genome combination was only found to be stable for grain yield per plant. The present results indicated that stability for different characters was not uniformly distributed in the four different genome combinations. This may be due to the distribution of genes in different genomes and to the interaction between them. The results also indicated similarities for two characters (100-grain weight and grain yield per plant) in the stability level of the four genome combinations. All four genome combinations were unstable for 100-grain weight and were stable for grain yield per plant. This non-specificity of the genome stability for these two characters may be due to similar selection criteria being followed in all the four groups, leading to the selection of similar genes. With regard to grains per spike the genome combinations AABB and AABBDD were found to be stable, while the genome combinations AABBRR and AABBDDRR were unstable. This unstability could be due to the addition of R-genome chromosomes.

Table 5

Comparative study of stability of different genome combinations for three characters

Characters	AABB	AABBDD	AABBRR	AABBDDRR
Grains per spike	Stable	Stable	Unstable	Unstable
100-grain weight	Unstable	Unstable	Unstable	Unstable
Grain yield/plant	Stable	Stable	Stable	Stable

Although for some characters genome specificity for stability may be present as indicated above, the stability depends largely on gene combinations rather than on the genome combination, as the presence or absence of the gene combination depends on the selection history of the genotypes irrespective of the genome combination.

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GENERATION MEAN ANALYSIS OF DROUGHT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.)

E. FARSHADFAR, M. GHANADHA[1], M. ZAHRAVI and J. SUTKA[2]

COLLEGE OF AGRICULTURE, RAZI UNIVERSITY, KERMANSHAH, IRAN
[1]COLLEGE OF AGRICULTURE, TEHRAN UNIVERSITY, TEHRAN, IRAN
[2]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY

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To evaluate the genetic background of quantitative criteria of drought tolerance in wheat, six generations of a cross between the varieties of Plainsman and Cappelle Desprez were grown in a randomized complete block design with three replications in the greenhouse of the College of Agriculture of the University of Tehran in 1997.

Genetic variation was found for yield potential (Yp), stressed yield (Ys), excised leaf water retention (ELWR), relative water loss (RWL), relative water content (RWC) and harvest index (HI) under water stress conditions.

High heterosis and heterobeltiosis were observed in the F_1 hybrid for Ys, HI and spike yield index (SYI). Genetic analysis exhibited overdominance in the inheritance of Ys, RWL, ELWR, HI, biomass and SYI, while RWC and Yp were controlled by the additive type of gene action. High narrow-sense heritability estimates were shown by ELWR, biomass and SYI. The high genetic advance for ELWR, RWC, HI and SYI indicated that direct selection could be effective for these traits. The epistatic effects (additive × additive=[i] for Yp, Ys and RWL, additive × dominance=[j] for ELWR, and dominance × dominance =[*l*] for RWL) were found to be outstanding.

Key words: wheat (*T. aestivum* L.), drought tolerance, stress tolerance index, gene action, scaling test

Introduction

Among the environmental stresses drought is the second contributor to yield reduction after disease (Farshadfar et al., 1995; Kristin et al., 1997). To improve the drought resistance of a crop, the most important step is to understand the mode of inheritance, the magnitude of gene effects and their mode of action (Bushuk et al., 1989; Acevedo and Ceccarelli, 1989; Snape, 1987). Many workers developed genetic models for the estimation of different genetic effects (Gamil and Saheal, 1986; Kearsey and Pooni, 1996). A comprehensive review of the evaluation of the genetic components of variation was presented by Haluver and Miranda (1985). In most of these methods only one generation is employed, but in generation mean analysis, the mean of different generations is used to calculate genetic effects (Haluver and Miranda, 1985). A general method of testing the expected relationships between generations using an additive dominance model was proposed by Cavalli (1952) and illustrated by Mather and Jinks (1982). A significant difference was found for the drought tolerance criteria wheat varieties Plainsman (drought-tolerant) and Cappelle Desprez (drought-sensitive) in various studies (Galiba et al., 1989 ; Farshadfar et al., 1993). These two parents and their subsequent generations were thus selected for the genetic analysis of drought tolerance. The objective of the present investigation was the genetic analysis of quantitative indices of drought tolerance in wheat using generation mean analysis.

Materials and methods

The experimental material consisted of six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) derived from crosses between Plainsman and Cappelle Desprez in the Agricultural Research Institute of the Hungarian Academy of Sciences in 1992. The parents (P_1 and P_2), the first (F_1) and second (F_2) generation hybrids and the first ($P_1 \times F_1= BC_1$) and second ($P_2 \times F_1=BC_2$) backcrosses were grown in a randomized complete block design with three replications in the greenhouse of the College of Agriculture of the University of Tehran in 1997. From each generation 5 seeds were selected and placed in Petri dishes containing Vitavax (2/1000) solution for vernalization.

The Petri dishes were transferred to low temperature (4°C) for 5 weeks, after which the seeds were sown into pots.

Each pot contained 3–5 seeds. The experiment was conducted under two different water regimes (irrigated and water stress). The pots in both the irrigated and water-stressed treatments were irrigated every day at the beginning of the experiment. After the emergence of 50% of the spikes, the pots in the water stress treatment received no more water until harvesting. Five plants were randomly selected from the water stress conditions and the following characteristics were measured:

1. Rate of water loss (RWL): The youngest leaf of each plant was collected and weighed. The leaves were then wilted at 30°C and reweighed, transferred to the oven for 24 h and weighed again. RWL was calculated using the formula suggested by Yang et al. (1991):

$$\mathrm{RWL} = (W_1 - W_2 / W_3)\ (t_1 - t_2 / 60)$$

where W_1, W_2 and W_3 are initial, wilted and dried weights respectively, t_1 and t_2 are the time of measurement for initial and wilted weight (in minutes).

2. Excised leaf water retention (ELWR): The youngest leaves were collected and weighed, left for 5 hours, then wilted at 30°C and reweighed. ELWR was calculated using the following formula:

$$[1- (\text{weight of new leaves} - \text{weight of leaves after 5 hours}) / \text{weight of new leaves}] \times 100$$

3. Relative water content (RWC): A 4 cm segment of the youngest leaf was taken and cut into 2 cm segments and weighed (fresh weight = FW). Then the segments were placed in distilled water for 4 hours and reweighed to obtain turgor weight (TW). Thereafter the leaf segments were oven dried and weighed (dried weight=DW). RWC was calculated using the formula of Ritchie et al. (1990):

$$\%\,\mathrm{RWC} = [(\mathrm{FW} - \mathrm{DW}) / (\mathrm{TW} - \mathrm{DW})] \times 100$$

4. Grain yield: Five single plants were randomly selected from the irrigated and water stress experiments to obtain the yield potential (Yp) and stress yield (Ys), respectively.

5. Spike yield index (SYI) was calculated as: grain yield of single plant/spike weight.

6. Harvest index (HI) was obtained as: grain yield of single plant/shoot weight.

7. Biomass: the total weight of the plant was obtained as the biological yield (biomass).

8. Stress tolerance index (STI) was calculated using the formula suggested by Fernandez (1992):

$$STI = (Y_P)(Y_S)/(Y_P)^2$$

where Y_p is the potential yield (irrigated) and Y_S is the stress yield.

The statistical analysis involved analysis of variance and mean comparison of the characters measured using the statistical softwares MINITAB and SAS. The data obtained for the 6 generations P_1, P_2, F_1, F_2, BC_1 and BC_2 were analysed using the genetic model of Mather and Jinks (1982) as:

$$Y = m + ad + \beta h + \alpha^2{}_i + 2\,\alpha_i\beta_j + \beta^2{}_1$$

where Y = mean of one generation, m = mean of all generations (population mean), d = total additive effects, h = total dominance effects, i = total additive × additive effects, j = total additive × dominance effects , l = total dominance × dominance effects and α, $2\alpha\beta$ and β^2 are the coefficients of the model parameters.

To estimate the parameters and to select the most suitable model the least squares method and the joint scaling test of Mather and Jinks (1982) were employed. Broad-sense and narrow-sense heritabilities were estimated using the formulae reported by Warner (1952):

$$H_b = (VF_2 - VE) / VF_2$$

$$\text{and } Hn = [2VF_2 - (VBC_1 + VBC_2)] / VF_2$$

Genetic advance was calculated (Allard, 1960) with a selection intensity of i=5% for all the characters as:

$$G_A = i \,.\, H_b \,.\sqrt{VF_2}$$

The components of variation for the six generations were calculated by the formulae of Mather and Jinks (1982) as:

$$E_W = 1/4\,(VP_1 + VP_2 + 2VF_1)$$

$$D = 4VF_2 - 2(VB_1 + VB_2)$$

$$H = 4\,(VB_1 + VB_2 - VF_1 - VE)$$

$$F = VB_2 - VB_1$$

where E_W = environmental variance effects, D = additive variance effects, H = dominance variance effects and F = joint contribution (association) on all the loci.

Results and discussion

The results of analysis of variance for the characters under investigation are presented in Table 1.

Table 1

Analysis of variance for the characters used for the genetic analysis of drought tolerance

S.O.V	df	Mean squares							
		Yp	Ys	RWL	RWC	ELWR	SYI	HI	Biomass
Generations	5	0.1815**	0.0591*	0.46(*)	640.7*	118**	33.04^{ns}	101.6(*)	0.45^{ns}
Replication	2	0.0184^{ns}	0.0135^{ns}	0.82*	131^{ns}	475(*)	60.3^{ns}	16.9^{ns}	0.076
Error	10	0.0237	0.0158	0.155	159	154	25.2	36.5	0.19

**, * and (*): Significant at 0.01, 0.05 and 0.1 probability levels, respectively; ns = non-significant

The analysis of variance revealed that all the generations had highly significant differences for yield potential (Y_p) and excised leaf water retention (ELWR). A significant difference was also found for stress yield (Y_s), relative water loss (RWL), relative water content (RWC) and harvest index (HI). No significant difference was found between the generations for biomass and spike yield index (SYI). There were significant differences between the generations for Y_p, Y_s, RWL, RWC, ELWR and HI, indicating the existence of variation, genetic distance and the possibility of selection for drought tolerance between the parents. Genetic variation was found in wheat for ELWR and RWL by Bayles et al. (1937) and Dedio (1975), for RWC by Manette et al. (1988), and for HI and grain yield by Ehdaie and Waines (1994).

The results of mean comparison (Table 2) indicated that Plainsman, which had the highest Y_p, showed a significant difference from Cappelle and BC_2. The F_1 hybrid had the highest Ys, HI, biomass and SYI and exhibited good heterosis over the midparents. BC_1 also displayed satisfactory heterosis for Y_p, Y_S, ELWR, RWC, HI, biomass and SYI over the midparents. The outstanding performance of the F_1 hybrid and BC_1 could be due to the proportion of Plainsman. The significant genetic distance between Plainsman and Cappelle for drought tolerance criteria was confirmed by Galiba et al. (1989) and Farshadfar et al. (1993).

Transgressive segregation was observed for ELWR in the F_2 generation. The highest stress tolerance index was revealed by the F_1 hybrid (STI=1.13) (Table 3), displaying the presence of heterobeltiosis for drought resistance in the F_1 hybrid. The lowest STI observed for Cappelle (STI=0.13) showed the drought sensitivity of Cappelle, which is supported by the results of Galiba et al. (1989) and Farshadfar et al. (1993). A three-dimensional plot (Fernandez, 1992) of Y_p, Y_S and STI (Fig. 1) was used to separate the group A generations (with high Y_p, Y_S and STI) from the group B generations (with high Y_p), group C generations (with high Y_S) and group D generations (with low Y_S and Y_p). The three-D plot put the F_1 hybrid in group A, with satisfactory heterobeltiosis for drought tolerance.

Table 2
Mean comparison of the characters studied

Generations	Characters							
	Y_p	Y_S	RWL	ELWR	RWC	HI	Biomass	SYI
Plainsman (P)	A 0/76	B 0/30	A 0/95	C 42/03	A 78/63	A 24/92	AB 1/19	A 54/22
Cappelle (C)	C 0/22	B 0/17	A 0/97	C 46/23	B 63/74	B 11/42	AB 1/19	A 37/52
F_1 (P×C)	AB 0/64	A 0/52	A 0/82	BC 51/67	AB 76/25	A 26/21	A 1/98	A 49/49
F_2	AB 0/70	B 0/19	AB 0/68	AB 61/65	A 79/04	AB 19/25	B 0/92	A 43/73
P×F_1 (BC_1)	AB 0/60	AB 0/31	B 0/31	A 74/42	A 82/54	A 23/26	AB 1/35	A 49/11
C×F_1 (BC_2)	BC 0/38	B 0/14	AB 0/14	AB 43/53	A 85/83	AB 15/32	B 0/87	A 35/51

Table 3
Yield potential (Y_p), stress yield (Y_S) and stress tolerance index (STI) for each generation

Generations	Y_p	Y_S	STI
Plainsman (P)	0.7584	0.2974	0.76
Cappelle (C)	0.2219	0.1730	0.13
F_1 (P × C)	0.6420	0.5239	1.13
F_2	0.4012	0.1940	0.46
F_1×P (BC_1)	0.6040	0.3087	0.64
BC_1× P (BC_2)	0.3775	0.1408	0.18

The degree of dominance (h/d), broad-sense (H_b) and narrow-sense (H_n) heritabilities, genetic advance (G_A) and genetic components of variation are presented in Table 4, which shows that the degree of dominance (h/d) for Ys, RWL, ELWR, HI, biomass and SYI was greater than one, indicating the presence of the overdominance type of gene action in the inheritance of these traits. Selection of these characters must therefore be delayed until the F_3 or F_4 generation. This delay permits a loss of non-additive genetic variance through inbreeding, so that the additive genetic variance can be more clearly evaluated.

Relative water content (RWC) and yield potential (Yp) were controlled by the additive type of gene action. The existence of the additive type of gene action in the inheritance of grain yield and RWC was reported by Limin and Fowler (1991), El-Hennawy (1992) and Manette et al. (1988).

As Yp and RWC are controlled by the additive type of gene action, the pedigree method of selection can be used for the improvement of these traits, while for characters under the control of the non-additive type of gene action, biparental mating offers good prospects for increasing the frequency of genetic recombination, hastening the rate of genetic improvement, though it may be necessary to resort to heterosis breeding (Gill et al., 1972; Sharma and Singh, 1976; Srivastava et al., 1980).

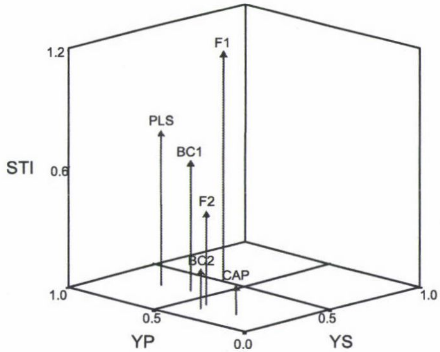

Fig. 1. Three dimensional plot of STI, YP and YS for the six generations of wheat

Table 4
Genetic parameters and components of variation for the drought tolerance criteria investigated

Characters	h/d	H_b	H_n	G_A	D	H	F	E_W	$\sqrt{H/D}$	$F/\sqrt{H \times D}$
Y_p	+0.57	0.58	0.51	0.33	0.20	0.01	+0.05	0.00	0.21	1.20
Y_S	+4.64	0.31	0.25	0.09	0.05	0.01	–0.03	0.01	0.40	–1.42
RWL	–12.80	0.57	0.12	0.11	0.05	0.54	+0.02	0.06	3.28	+0.14
ELWR	+3.58	0.74	0.73	21.56	299.68	9.15	–17.05	53.53	0.18	–0.33
RWC	+0.68	0.62	0.24	5.92	68.88	119.47	–5.12	79.20	1.32	–0.06
HI	+1.19	0.88	0.35	7.18	70.39	223.25	+5.32	8.04	1.78	+0.04
Biomass	+3.61	0.94	0.91	1.18	0.51	0.41	+0.18	0.04	0.89	+0.40
SYI	+3.00	0.86	0.85	13.69	104.32	0.99	+21.99	8.75	0.10	+2.16

The variation exhibited by the characters investigated showed that selection for these traits may be effective in the improvement of drought tolerance. However, the selection efficiency is related to the magnitude of heritability and genetic advance (Johnson et al., 1955). Low narrow-sense heritability was observed for RWL, but RWC, Ys and HI exhibited moderate H_n. The rest of the characters displayed high heritability. The low H_n in RWL suggests that environmental effects constitute a major portion of the total phenotypic variation for this character (Farshadfar, 1995). The high heritability estimates for Yp, ELWR, RWC, biomass and SYI showed that effective progress can be made through selection. High heritability was observed for RWC (Manette et al. 1988), biomass (Farshadfar et al., 1993), Ys and HI (Ehdaie and Waines, 1994). The difference between H_n and H_b for RWL, HI and RWC exhibits the involvement of the dominance effect in the genetic constitution of these characters.

The rate of genetic advance is connected with heritability (Mather and Jinks, 1982). The genetic advance for Ys was lower than that of Yp, indicating the significance of indirect selection for Ys through correlated characters with high heritability and genetic advance. The same conclusion can be made for RWL and biomass, while direct selection may be more effective for ELWR, RWC, HI and SYI, because of the high genetic advance observed for these traits.

The value of F for Yp, RWL, HI and SYI was positive, hence most of the genes responsible for these traits are dominant in Plainsman, while the F value for Ys, ELWR and RWC was negative, indicating that most of the genes controlling these characters are dominant in Cappelle Desprez.

The joint scaling test (Mather and Jinks, 1982) was employed to estimate the mean [m], additive effect [d], dominance effect [h], additive × additive [i], additive × dominance [j] and dominance × dominance [l] values. The best dominance-additive model was selected using the non-significant chi-square test (χ^2) and the lowest standard error (Table 5).

Table 5

Estimates of the genetic components of the mean for the characters studied

Characters	m	[d]	[h]	[i]	[j]	[l]	χ^2
Yp	0.49±0.002**	–0.27±0.02**	0.15±0.03**	–0.15±0.03**	–	–	1.20ns
Ys	0.64±0.01**	0.07±0.02**	0.72±0.18**	0.43±0.16**	–	–	0.99ns
RWL	0.94±0.06**	–0.35±0.06ns	–1.33±0.44**	–	–	1.27±0.42**	3.14ns
ELWR	44.13±1.5**	–2.10±1.55ns	82.90±1.71**	–	26.08±13.06*	–75.37**±11.86	1.63ns
RWC	79.39±2.09**	7.22±1.42**	34.43±11.27*	–8.04±2.62**	–	–	5.77ns
HI	17.88±38**	6.85±1.39**	8.13±1.82**	–	–	–	0.84ns
SYI	42.28±1.55**	3.24±1.15**	10.84±2.07**	–	–	–	–

*,**: Significant at 0.05 and 0.01 levels of probability, respectively; ns: non-significant

The genetic models fitted for Y_p, Y_S and RWC indicated dominance and additive × additive gene effects (Table 5). It is therefore suggested that selection should be carried out in later generations and the interaction should be fixed by selection under selfing conditions. No significant additive effect [d] was found for RWL and ELWR, indicating that selection is not effective in early generations. The epistatic effect (additive × dominance = [j]) was significant for ELWR, which is not fixable by selection under selfing conditions. Both additive × dominance [h] and dominance × dominance [l] effects were significant for RWL and ELWR, supporting the presence of the duplicate type of epistasis. This complementary interaction increases the variation between the generations and in the segregating population. The significance of [d] and [h] in the inheritance of HI and SYI revealed that both types of additive and dominance effects are involved in the genetics of harvest index and spike yield index. As there was no significant difference between the generations for biomass, the Chi-square test was significant for most of the fitted models and hence the components were not significant for this trait.

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PRODUCTION OF NEW TETRAPLOID TRITICALE FORMS

S. ARUMUGAM and V. R. K. REDDY

CYTOGENETICS LABORATORY, DEPARTMENT OF BOTANY, BHARATHIAR UNIVERSITY, COIMBATORE, INDIA

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Attempts were made to produce tetraploid triticales by crossing 6x triticales with diploid rye. In F_2, the chromosome number was reduced to between 15 and 23 except in three plants, where the chromosome number was 28, 32 and 38, respectively. An increased frequency of ring bivalents was observed in many F_4 plants. In the progeny of the plant with 28 chromosomes, desired plants (four) with 2n = 28 chromosomes were obtained. Data on various agronomic characters were recorded on the progeny of these plants in F_5. Reasonably good fertility was noticed in these tetraploid triticale forms.

Key words: tetraploid triticale, cytology, agronomic characters

Introduction

The development of polyploid forms is recognized as one of the pathways of plant evolution. Triticales have been synthesized in the past at four different ploidy levels, namely 4x, 6x, 8x and 10x. The development of 4x triticale species from crosses between 6x triticale and rye appears to be an example which will help in the understanding of biological events leading to a new genomic structure. Attempts to use tetraploid triticale in practical breeding have failed because of the low yield of 50–60% compared with hexaploid. Today the use of 4x triticale is mainly seen as a tool in improving hexaploid triticale and as an object for studying aspects of the evolution of a new polyploid. Tetraploid triticales have lower aneuploid frequency than the octoploid and hexaploid triticales and may prove useful in the improvement of hexaploid triticales. They are also likely to provide a better insight into the interaction of different chromosomes of the A and B genomes with a complete complement of rye. Since tetraploid triticales have only been synthesized in a limited number, there is a need to synthesize them in larger numbers to provide better variability for breeding programmes. The present communication reports the production of new fertile tetraploid triticale forms.

Materials and methods

Four hexaploid triticales, namely Borba, Carman, Currency and Venus, were crossed with diploid rye (Petkus rye) in order to produce 4x triticales. The cytology of hybrids/plants in different generations (F_1, F_2, F_3 and F_4) was studied. Data on various quantitative agronomic characters of the 4x triticales thus produced were recorded.

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Results and discussion

The number of chromosomes in F_1 hybrids ranged from 27 to 29 (Table 1). Most of the plants had 7 bivalents and 14 univalents (Fig. 1a), as expected. In some cases the number of ring bivalents was low as compared to rod bivalents, and trivalents were frequently found. The chromosome number in F_2 plants was reduced to between 15 and 23 (Figs 1b–1f), except in three plants, T-29(6)1, T-29(6)2 and T-29(6)4, where the chromosome number was 28, 32 and 38 respectively (Fig. 1e; Table 2). An increased frequency of bivalents was noticed in these three plants, while in all the others there were fewer bivalents per cell relative to F_1. Seeds could be obtained only from two F_3 plants, T-29(6)1 and T-29(6)4 (Table 3). Two plants from T-29(6)4 and four plants from T-29(6)1 were grown to maturity. All the six F_4 plants were studied cytologically. The chromosome numbers were 2n = 44 and 39, respectively (Table 4) in the two plants descended from T-29(6)4, while all the four plants derived from T-29(6)1 showed 2n = 28 chromosome number (Fig. 1f). Good seed set was obtained from the F_4 plants. A chromosome number of 2n = 28 was cytologically confirmed in the F_5 plants. Data on various agronomic characters were recorded on all the F_5 plants obtained (Table 5).

Table 1

Chromosome associations at meiotic metaphase I of F_1 hybrids involving 6*x* triticales and 2x rye

Plant number	2n	I	II			III
			Total	Ring	Rod	
T-11(2)	28	12–20 (16, 15.84)	4–8 (6. 6.08)	1–4 (2, 1.80)	3–7 4.28)	–
T-25(1)	28	12–20 (16, 15.95)	3–8 (6, 6.08)	0–3 (1, 1.30)	1–7 (4, 4.58)	0–1 (0, 0.15)
T-27(1)	28	11–18 (14, 15.25)	4–7 (7, 6.15)	0–3 (1, 1.40)	3–7 (5, 4.75)	0–1 (0, 0.15)
T-27(3)	27	12–17 (15, 14.92)	5–8 (6, 5.92)	0–3 (1, 1.32)	2–6 (5, 4.60)	0–1 (0, 0.08)
T-27(8)	28	9–17 (14, 13.76)	4–9 (7, 7.00)	1–5 (4. 3.76)	1–5 (3, 3.24)	0–1 (0, 0.08)
T-27(10)	28	12–16 (14, 14.40)	6–8 (7, 6.80)	0–4 (1. 1.80)	3–7 (6, 5.00)	–
T-27(11)	29	9–17 (13, 12.32)	6–10 (8, 7.68)	2–5 (4. 3.88)	1–8 (4, 3.80)	0–1 (0, 0.44)
T-27(13)	28	10–17 (12, 13.60)	4–9 (6, 6.30)	2–3 (3. 2.50)	1–7 (3, 3.80)	0–2 (0, 0.60)
T-29(6)	28	8–18 (12, 13.60)	5–10 (8, 7.96)	1–6 (3, 3.04)	2–8 (4, 4.92)	–
T-29(8)	28	14–18 (14, 15.00)	5–7 (7, 6.50)	0–5 (3, 2.10)	1–7 (4, 4.40)	–
T-29(9)	28	13–18 (18, 15.60)	5–7 (5, 5.90)	0–1 (0, 0.30)	4–7 (5, 5.60)	0–1 (0, 0.20)

In each column, the figures in the first row are the range and those in the second row, in parentheses, are the mode and mean, respectively.

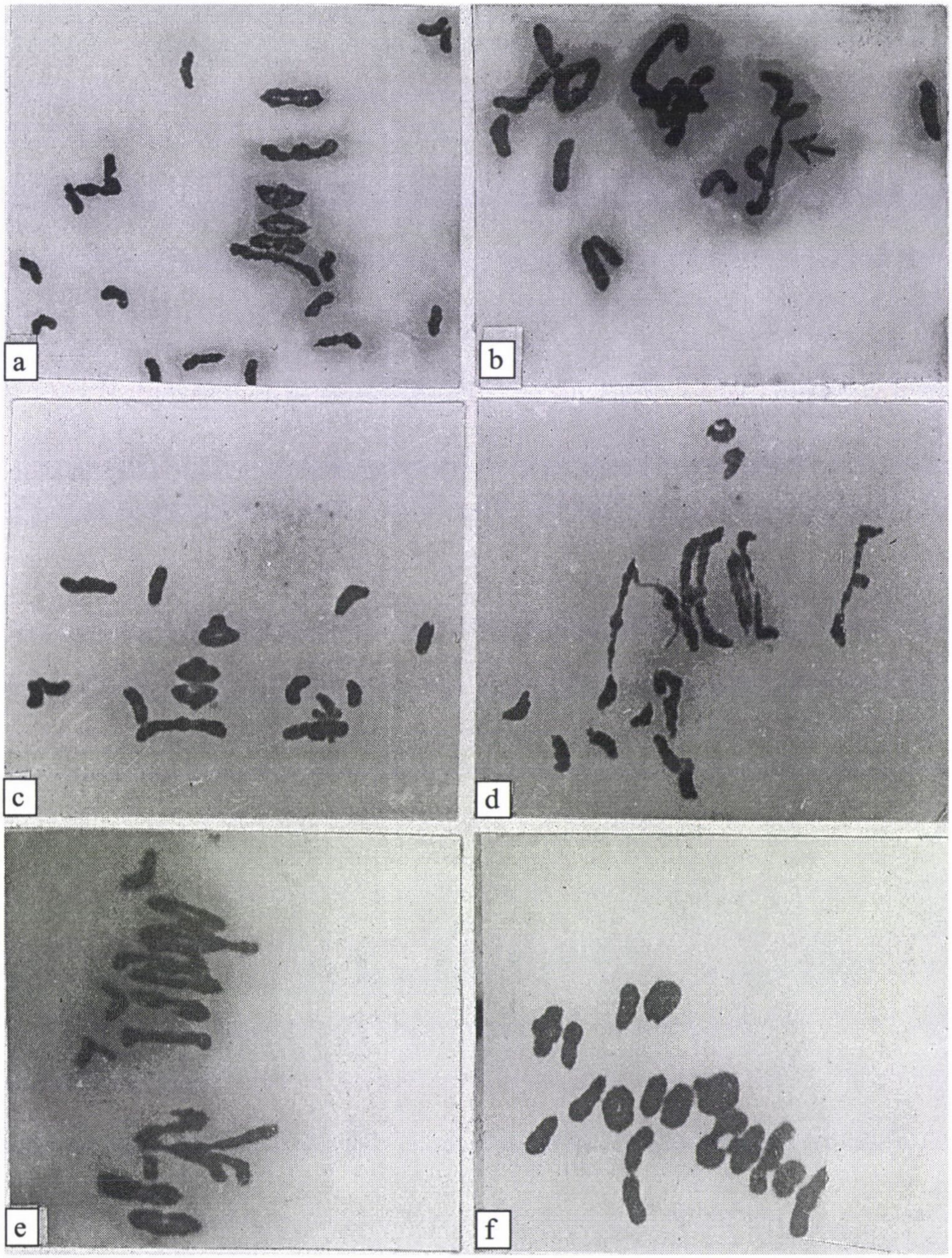

Fig. 1. Cytology of F_1, F_2 and F_4 hybrids derived from 6x triticale × 2x rye. a: Metaphase I of the F_1 plant T-29 (6) with $7^{II}+14^{I}$; b: Metaphase I of the F_2 plant Tr-8 G4(2) (2n=20) with $6^{II}+8^{I}$; c: Metaphase I of F_2 plant Tr-8 G1(1) (2n=22) with $4^{II}+14^{I}$; d: Metaphase I of F_2 plant Tr-8 G4(3) (2n=23); e: Metaphase I of F_2 plant T-29 (6) 1 (2n=28) with $11^{II}+1^{III}+3^{I}$; f: Metaphase I of F_4 plant T-29(6)1(1)1 (2n=28) with $13^{II}+2^{I}$

Table 2
Chromosome associations at meiotic metaphase I of F_2 segregants involving 6x triticale and 2x rye

Plant number	2n	I	II			III	IV
			Total	Ring	Rod		
T-27(3)3	20	6–14 (8, 8.60)	3–7 (6, 5.70)	0–3 (0, 0.60)	3–7 (6, 5.10)	–	–
T-27(3)4	20	14–20 (20, 18.13)	0–3 (0, 0.93)	–	0–3 (0, 0.93)	–	–
T-29(6)1	28	0–7 (2, 2.80)	7–14 (11, 1.40)	7–9 (6, 7.80)	2–6 (3, 4.14)	0–1 (0, 0.02)	–
T-29(6)2	32	6–12 (8, 8.24)	2–15 (13, 13.44)	8–12 (9, 9.96)	2–6 (1, 3.82)	0–1 (0, 0.02)	–
T-29(6)3	21	5–9 (7, 7.52)	4–8 (6, 5.88)	0–6 (2, 2.48)	1–6 (3, 3.40)	0–1 (0, 0.36)	0–1 (0, 0.16)
T-29(6)4	38	4–14 (8, 7.13)	12–17 (15, 15.17)	6–12 (10, 9.73)	2–9 (5, 5.43)	–	0–1 (0, 0.13)
T-29(6)5	22	5–18 (10, 9.44)	2–8 (6, 5.76)	0–4 (1, 1.44)	2–6 (5, 4.32)	0–1 (0, 0.24)	0–1 (0. 0.08)
T-29(6)6	23	11–19 (15, 15.04)	2–6 (4, 3.72)	0–3 (0. 0.64)	1–5 (3, 3.08)	0–1 (0, 0.12)	0–1 (0, 0.04)
T-29(6)7	23	3–17 (7, 7.52)	3–10 (6, 6.29)	0–6 (4, 3.19)	1–7 (2, 3.09)	0–1 (1, 0.52)	0–1 (0, 0.33)
T-29(9)3	22	6–18 (10, 11.50)	1–8 (3, 4.60)	0–3 (1, 1.00)	0–8 (3, 3.60)	0–2 (0, 0.20)	0–1 (0, 0.10)

In each column, the figures in the first row are the range and those in the second row, in parentheses, are the mode and mean, respectively.

Tetraploid triticale, selected from the offspring of hexaploid triticale crossed with rye, has existed for about 25 years (Krolow, 1973), but its importance is still uncertain. The great potential for variations in the constitution of the wheat genome components, thus allowing for the breeding of numerous different forms, is well known (Lukaszewski et al., 1984; Krolow and Lukaszewski, 1986; Bernard and Bernard, 1987; Baum and Lelley, 1988; Badaev et al., 1992). The addition of mixed A and B genome chromosomes to rye to yield 4x triticales has been attempted on a limited scale. The tetraploid triticales thus synthesized have a complete complement of rye chromosomes (14) and mixed chromosomes for the A and B genomes (AxAx ByByRR, where $x + y = 7$; $2n = 28$).

In crosses between hexaploid triticale and diploid rye, most of the plants exhibited $7^{II} + 14^{I}$, as expected. The number of chromosomes in F_1 hybrids ranged from 27 to 29. The appearance of plants with 27 and 29 chromosomes may be due to the nullisomic and disomic pollens, respectively, expected to be produced in triticale due to meiotic instability. The frequency of ring bivalents in these hybrids was rather low, although the chromosomes mostly form ring bivalents in rye. Similarly, in some cases the number of bivalents exceeded seven. The reasons for this have been discussed earlier. Trivalents in F_1 hybrids may be due to wheat-rye translocation in the triticale parent. The chromosome

Table 3
Chromosome associations* at meiotic metaphase I of F_2 segregants involving 6x triticale and 2x rye

Plant number	2n	I	II		III	IV
			Ring	Rod		
Tr-8 G1(1)	22	9.80 (5–18)	1.56 (0–5)	4.48 (2–6)	0.04 (0–1)	–
Tr-8 G1(2)	20	7.20 (6–10)	2.20 (0–5)	4.20 (2–6)	–	–
Tr-8 G3(3)	21	7.52 (5–9)	1.32 (0–3)	5.36 (3–7)	0.04 (0–1)	–
Tr-8 G4(1)	21	6.12 (3–9)	4.12 (0–7)	2.88 (0–6)	0.24 (0–1)	0.04 (0–1)
Tr-8 G4(2)	20	7.40 (4–10)	3.96 (0–6)	2.28 (0–6)	0.04 (0–2)	–
Tr-8 G4(3)	23	6.32 (3–11)	2.50 (0–4)	4.68 (2–8)	0.36 (0–1)	0.24 (0–1)
Tr-8 G5(1)	21	10.28 (5–13)	1.08 (0–2)	4.20 (2–6)	0.08 (0–1)	–
Tr-8 G6(1)	21	7.08 (3–11)	3.16 (1–6)	3.56 (0–6)	0.16 (0–1)	–
Tr-8 G8(1)	21	7.12 (3–11)	3.28 (2–6)	3.68 (0–6)	–	–
Tr-8 G12(1)	21	7.80 (5–11)	3.76 (1–6)	2.84 (1–5)	–	–
Tr-22 G3(1)	19	13.48 (9–17)	0.08 (0–1)	2.68 (1–5)	–	–
Tr-22 G3(2)	21	9.28 (7–13)	2.72 (0–5)	3.08 (1–6)	0.04 (0–1)	–
Tr-22 G4(3)	15	6.64 (5–13)	0.36 (0–2)	3.76 (1–6)	0.04 (0–1)	–

*(mean from 25 p.m.c's); In each column, the figures in the first row are the mean values and those in the second row, in parentheses, are the range.

number in F_1 hybrid T-27(11) was found to be 29; this may be trisomic for one of the rye chromosomes, the extra chromosome being contributed by the triticale parent.

The chromosome number was reduced in the F_2 plants, except in three plants, T-29(6)1, T-29(6)2 and T-29(6)4. There were fewer bivalents per cell relative to F_1, except in T-29(6)1, T-29(6)2 and Tr-29(6)4. An increased frequency of ring bivalents was observed in the F_2 plants. This indicates that in most of the plants there was a reduction in the number of rye chromosomes. This tendency for a reduction in the chromosome number was also observed by Krolow (1974), who described it as a characteristic of hybrids of 6x triticale with (AB)(AB)RR tetraploid forms. This is unfortunate, because for the successful synthesis of tetraploid triticale seven pairs of rye chromosomes should be retained. These plants were selfed to achieve the objective of synthesizing tetraploid triticales with 14 bivalents. The frequency of univalents was usually high. In T-27(3)4 and Tr-22 G3(1), bivalents were almost absent at metaphase I,

Table 4

Chromosome associations at meiotic metaphase I of F_4 plants involving 6x triticale × 2x rye

Plant number	2n	I	II			III	IV
			Total	Ring	Rod		
T-29(6) 4(1)1	44	2–12 (8, 6.76)	15–21 (18, 17.72)	9–17 (12, 12.24)	3–10 (5, 5.48)	0–2 (0, 0.44)	0–1 (0, 0.12)
T-29(6) 4(6)1	39	4–9 (7, 6.20)	16–17 (16, 16.28)	10–13 (11, 11.32)	4–6 (5, 4.96)	0–1 (0, 0.08)	–
T-29(6) 1(1)1	28	0–4 (2, 2.48)	12–14 (13, 12.56)	7–11 (8, 9.34)	3–5 (4, 4.20)	0–1 (0, 0–1)	–
T-29(6) 1(1)2	28	0–6 (3, 2.60)	11–14 (10, 12.46)	7–10 (8, 9.36)	3–5 (4, 3.65)	0–1 (0, 0–1)	–
T-29(6) 1(1)3	28	0–4 (2, 2.80)	10–14 (11, 12.80)	6–11 (8, 9.78)	3–5 (4, 4.10)	0–1 (0, 0–1)	–
T-29(6) 1(1)4	28	0–4 (2, 2.64)	10–12 (11, 11.28)	7–11 (8, 9.86)	2–4 (3, 3.68)	0–1 (0, 0–1)	–

In each column, the figures in the first row are the range and those in the second row, in parenthesis, are the mode and mean, respectively.

Table 5

Data on various quantitative agronomic characters recorded on tetraploid triticale forms developed in the F_5 generation (in each column, the first row is the mean and the figures in second row, in parenthesis, are the range)

1	2	3	4	5	6	7	8	9	10	11	12
1	T-29(6) 1(1)1	13	57.46 (53–61)	4.20 (3–6)	13.53 (9–17)	35.23 (28–42)	128.20 (96–164)	52.48 (43–75)	1.46 (1.10–1.70)	12.32 (4–16)	2.42 (1.6–2.9)
2	T-29(6) 1(1)2	11	52.40 (49–55)	4.45 (3–7)	14.61 (11–19)	34.78 (27–40)	135.23 (98–157)	47.21 (38–67)	1.52 (0.89–1.82)	11.46 (3–15)	2.33 (1.7–2.8)
3	T-29(6) 1(1)3	9	61.72 (58–64)	3.73 (2–6)	15.22 (12–20)	33.46 (26–39)	124.67 (94–154)	49.67 (40–72)	1.25 (0.92–1.68)	13.48 (4–16)	2.48 (1.8–3.2)
4	T-29(6) 1(1)4	15	58.36 (56–63)	4.08 (3–6)	13.83 (9–18)	37.23 (29–43)	139.75 (89–162)	50.23 (39–83)	1.39 (0.89–1.74)	14.08 (4–17)	2.64 (2.0–3.4)

1: Sl No.; 2: 4x Triticale line (F_5); 3: No. of plants; 4: Plant height (cm); 5: Tiller number/plant; 6: Spike length (cm); 7: Spikelets/spike; 8: Florets/spike; 9: Seeds/spike; 10: Seeds/spikelet; 11: Grain yield (g); 12: 100-grain weight (g)

but some pairing was observed in early stages, indicating that the high univalent formation may be at least partly due to desynapsis. Besides the above, no satisfactory explanation for the failure of pairing can be given, although speculation is possible. For instance, it may be due to the loss of pairing promoter genes or due to mutations for reduced pairing in these hybrids. Multivalents were also observed in certain F_2 plants and might have resulted either due to homoeologous pairing or due to translocations.

Seeds could not be obtained from T-29(6)2. The number of chromosomes increased further in F_4 plants of T-29(6)4 (2n = 39 and 44), and the number of bivalents was 18 and 16, so they cannot yield 4x triticales on selfing. However, all the four F_4 plants from T-29(6)1 showed 2n = 28 chromosome number. Progenies of these plants also showed the same chromosome number in the F_5 generation, suggesting that stability has been achieved in these triticales.

From the foregoing discussion, it emerges that plants with an increased number of chromosomes could survive up to the F_4 generation, while those with a reduced number of chromosomes could not yield seeds beyond F_2. Hence, to facilitate better seed setting, such F_2 plants should be backcrossed to 6x triticales, in addition to being selfed as was done in the present study. Such a strategy would lead to many more progenies of F_2 plants, with two parallel descents, and would enhance the possibilities of obtaining tetraploid triticale. Such backcrosses were found to yield superior tetraploid triticale forms (Lehmann and Krolow, 1993; Stoinova and Sabeva, 1996).

The agronomic characters of the derived tetraploid triticale forms are reasonably good, although the yield remains low compared to hexaploid triticale. Low yield is mainly due to lower number of tillers per plant and small seeds coupled with low kernel weight. Nevertheless, this material offers great variability and a broad basis for selection. Improvement in these tetraploid triticales can be attempted by crossing hybrids of 6x auto-alloploid triticale and wheat with 4x triticale, which proved to be useful (Lehmann et al., 1991), as it not only facilitates the incorporation of genetic material from bread wheat but also results in great variability. Improved secondary tetraploid triticales were obtained in this way by crossing primary 4x triticale with 6x triticale (Sabeva and Stoinova, 1994). These tetraploid forms can also be used for the genome reconstruction of amphiploids with diverse ploidy levels.

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EFFECTS OF WATER SUPPLY AND SOWING DATE ON PERFORMANCE AND ESSENTIAL OIL PRODUCTION OF ANISE (*PIMPINELLA ANISUM* L.)

S. ZEHTAB-SALMASI[1], A. JAVANSHIR[1], R. OMIDBAIGI[2], H. ALYARI[1] and K. GHASSEMI-GOLEZANI[1]

[1]DEPARTMENT OF AGRONOMY AND PLANT BREEDING, FACULTY OF AGRICULTURE, TABRIZ UNIVERSITY, TABRIZ, IRAN
[2]DEPARTMENT OF HORTICULTURE, FACULTY OF AGRICULTURE, TARBIAT MODARRES UNIVERSITY, TEHRAN,IRAN

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Field and greenhouse experiments were carried out in order to determine the effects of water supply and sowing date on the performance and essential oil production of anise (*Pimpinella anisum* L.). The results indicated that mean leaf area (LA), specific leaf area (SLA), relative growth rate (RGR), relative water content (RWC), grain yield and essential oil decreased, while the root/shoot ratio and the oil percentage of the seeds increased when the available soil water decreased to below 80% in the greenhouse. It was concluded that for higher grain and essential oil production, anise must be sown early in the spring (April 4 to 16) in Tabriz. Water deficit during stem elongation and umbel appearance reduced anise growth, grain yield and oil production. Seeds harvested at the waxy stage had higher oil content than those harvested at earlier or later stages.

Key words: anise, *Pimpinella anisum,* water supply, water limitation, sowing date, essential oil

Introduction

Anise (*Pimpinella anisum* L.) is an annual medicinal plant which belongs to the *Apiaceae* family. The essential oil extracted from anise seeds has antiseptic, antispasmodic, carminative, digestive (Gangrade et al., 1989; Hornok, 1992; Chevallier, 1996) and fungicidic (Singh et al., 1998) effects. It is also used in perfumes, tooth pastes and the liquor industry (Hornok, 1992; Dizdaroglu and Balkan, 1996).

In order to produce medicinal plants it is necessary to determine the environmental factors under which they give higher yields and better quality (Yanive and Palevitch, 1982; Omidbaigi, 2000). One of the most important factors affecting plant growth and the production of secondary metabolites is the water supply (Randhawa et al., 1992). Hornok (1992) pointed out that insufficient water supply at seed germination and also from stem elongation until flowering can reduce the grain yield of anise by 150 to 200 kg ha^{-1}. It has also been reported that irrigation can increase the grain yield of anise without any effect on the essential oil content (Abdel-Kawy et al., 1981). Sowing date is another factor that affects anise grain yield and essential oil content (Fazekas et al., 1981; Maheshwari et al., 1984). Fazekas et al. (1981) showed that anise seeds sown in mid-March produced plants with higher grain yield.

Because of the medicinal importance of anise, the present research was aimed at quantifying the effects of water supply, sowing date and harvesting time on the performance and essential oil yield of the crop, which was grown for the first time in Tabriz, in the greenhouse and field.

Materials and methods

Seeds of anise (*Pimpinella anisum* L. cv. Soroksári), provided from Hungary, were used in this study. Greenhouse and field experiments were conducted at Tabriz University (38°5' N; 46°17' E; elevation 1362 m) during 1999–2000.

The greenhouse experiment was carried out in a randomized complete block design (RCBD) with four replications. The treatments were 20, 40, 60, 80 and 100% of available water (AW). These percentages of available water were kept constant in the soil by daily weighing and watering of the pots. Plant-free pots were used to estimate evaporation (Van den Boogaard, 1995). The soil was sandy-loam with 21% FC and 12% PWP.

In the field experiment five irrigation treatments were used: I_1 = irrigation was omitted during stem elongation (SE), I_2 = irrigation was omitted during the stem elongation and grain filling stages (SE + GF), I_3 = irrigation was omitted during umbel appearance (UA), I_4 = irrigation was omitted during the grain filling period (GF), and I_5 = plots were irrigated in all growing stages (control). These treatments were studied on three sowing dates (April 4, 16 and 29) in a factorial experiment. Plots of 5 rows, 0.32 m wide and 2.5 m long, were arranged in a randomized complete block design (RCBD) with three replications. The soil texture was sandy loam with 7.37 pH and 17% FC.

In both experiments the essential oil content was determined by water distillation of the samples using the Clevenger method (cited by Ondarza and Sanchez, 1990).

Results

The statistical analysis showed that all the characteristics studied were affected significantly by water treatments in the greenhouse ($P < 0.01$). However, reducing the water supply from 80% down to 20% available water resulted in a decrease in mean leaf area (LA), specific leaf area (SLA), relative growth rate (RGR) and relative water content (RWC) and an increase in the root/shoot (R/S) dry weight ratio (Table 1). For all traits except for R/S ratio, there were no significant differences between the control and 80% available water (Table 1).

Table 1

Effect of water supply on the means of growth parameters and grain yield of anise in the greenhouse

Treatments	LA (cm^2)	SLA ($cm^2 g^{-1}$)	RWC (%)	RGR (g/g/day)	R/S	Grain yield (g/pot)
100 % AW+	0.0650 a	143.3 a	0.887 a	0.0650 a	0.135 e	1.317 a
80 % AW	0.0630 a	141.7 a	0.879 a	0.0640 a	0.170 d	1.235 a
60 % AW	0.0502 b	121.7 bc	0.750 b	0.0535 b	0.200 c	1.002 c
40 % AW	0.0447 c	119.8 c	0.682 c	0.0467 c	0.237 b	0.822 c
20 % AW	0.0395 d	106.5 d	0.610 d	0.0400 d	0.295 a	0.647 d

+(control); *Values within columns followed by the same letter do not differ at $P < 0.05$, based on Duncan's new multiple range test.

The average grain yield was significantly different in the various water supply treatments, ranging from 0.647 g pot^{-1} in the 20% available water treatment to 1.317 g pot^{-1} at 100% available water (Table 1).

In the greenhouse, when the available water decreased to below 60%, the essential oil percentage increased, but the total amount of oil per plant decreased. The average percentage of essential oil and the mean oil production per pot ranged from 2.140% and 13.85 mg pot^{-1} (20% available water) to 1.788 % and 23.54 mg pot^{-1} (100%) (Fig. 1).

In the field experiment, yield components such as number of umbels, umbelets, flowers and 1000 grain weight were significantly decreased when sowing date was delayed and plants were exposed to water stress. However, harvest index (HI) increased at the latest sowing date and in the water deficit treatments (Table 2). Seeds sown on April 4 and 16 had greater biomass and grain yield than those sown on April 29. Average grain yield and biomass were reduced significantly by treatments I_1, I_2 and I_3, but the difference between I_4 and the control was not significant (Table 2 and Fig. 2).

The essential oil percentage of seeds in the field ranged from 2.216% to 2.753%. When the sowing date was delayed the percentage of oil was significantly decreased, but water limitation resulted in an increase in essential oil content (Table 2). Mean essential oil production under field conditions ranged from 10.12 to 23.74 kg ha^{-1} (Fig. 3).

In seeds harvested at the waxy stage (with 26 to 28% moisture), the essential oil content was higher than in seeds harvested earlier (in the milky stage) or later (in the ripening stage) (Fig. 4).

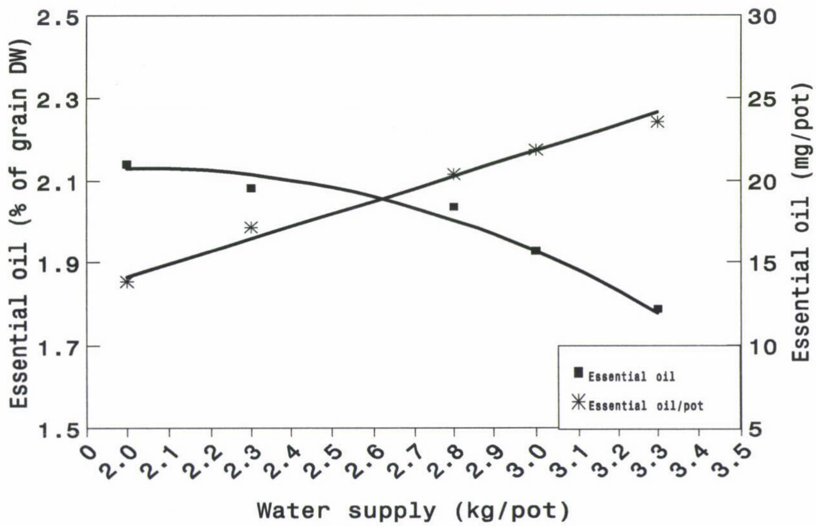

Fig. 1. Relationship of essential oil percentage and oil yield per plant with water supply. Predicted values of essential oil percentage were obtained by the equation $Y = 1.1941 + 0.9151\ X - 0.2237\ X^2$ ($r^2 = 0.956$). Predicted amounts of oil per plant were calculated using the equation $Y = 7.71371\ X - 1.32355$, ($r^2 = 0.996$)

Table 2

Mean values of several characteristics of anise affected by sowing date and water limitation in the field*

Sowing date	Water deficit	Leaf No.	Plant height, cm	Umbel No.	Umbelet No.	Flower No.	1000 GW (g)	Biomass (kg/ha)	HI (%)	Ess. oil (%)
D_1		–	–	–	15.85 a	11.67 a	2.408 a	–	39.67 b	2.753 a
D_2		–	–	–	14.08 b	10.47 b	2.295 b	–	39.61 b	2.744 a
D_3		–	–	–	12.54 c	9.49 c	2.199 c	–	40.31 a	2.216 b
	I_1	12.04 b	–	–	10.74 b	8.62 b	2.124 d	–	42.20 a	2.626 a
	I_2	11.81 b	–	–	10.79 b	8.56 b	2.017 e	–	42.67 a	2.646 a
	I_3	15.00 a	–	–	16.33 a	9.10 b	2.414 b	–	39.03 b	2.582 b
	I_4	14.94 a	–	–	16.29 a	12.97 a	2.307 c	–	37.83 c	2.523 c
	I_5	15.23 a	–	–	16.63 a	13.46 a	2.641 a	–	37.58 c	2.478 d
D_1	I_1	–	34.97 e	9.63 d	–	–	–	1487 e	–	–
	I_2	–	35.47 e	9.34 d	–	–	–	1422 ef	–	–
	I_3	–	43.37 b	17.37ab	–	–	–	1820 c	–	–
	I_4	–	44.27 ab	17.17ab	–	–	–	2254 ab	–	–
	I_5	–	44.97 a	17.57 a	–	–	–	2376 a	–	–
D_2	I_1	–	31.80 f	10.0 d	–	–	–	1421 ef	–	–
	I_2	–	31.77 f	10.30 d	–	–	–	1332 f	–	–
	I_3	–	41.97 c	16.30 b	–	–	–	1748 c	–	–
	I_4	–	43.60 b	16.20 b	–	–	–	2194 b	–	–
	I_5	–	44.00 b	16.27 b	–	–	–	2309 ab	–	–
D_3	I_1	–	30.50 g	8.23 e	–	–	–	1094 g	–	–
	I_2	–	31.00 fg	8.27 e	–	–	–	1032 g	–	–
	I_3	–	38.30 d	13.83 c	–	–	–	1323 f	–	–
	I_4	–	38.57 d	14.03 c	–	–	–	1608 d	–	–
	I_5	–	38.93 d	14.07 c	–	–	–	1696 cd	–	–

* Different letters represent significant differences at $P < 0.05$.

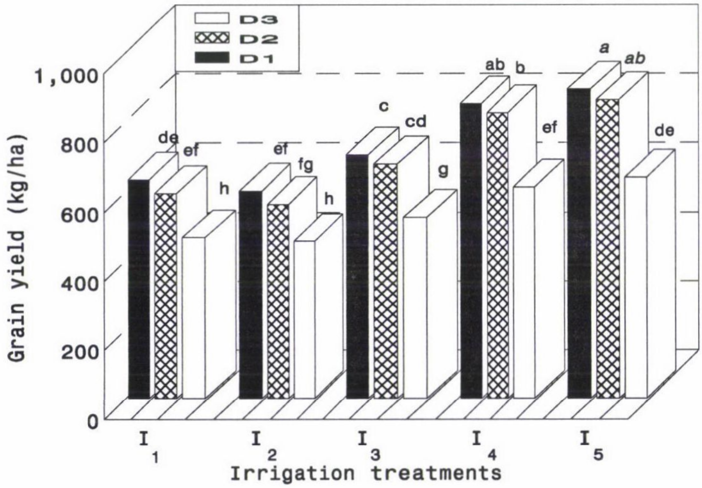

Fig. 2. Effects of sowing date and water limitation on mean grain yield of anise (*Pimpinella anisum* L.). Different letters indicate significant difference at $P < 0.05$

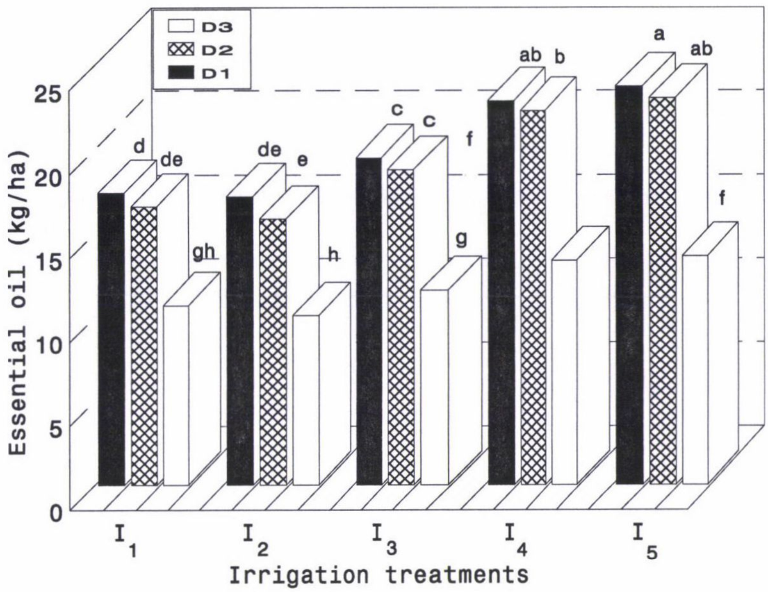

Fig. 3. Mean essential oil yield of anise affected by water limitation at different sowing dates. Different letters indicate significant differences at $P < 0.05$

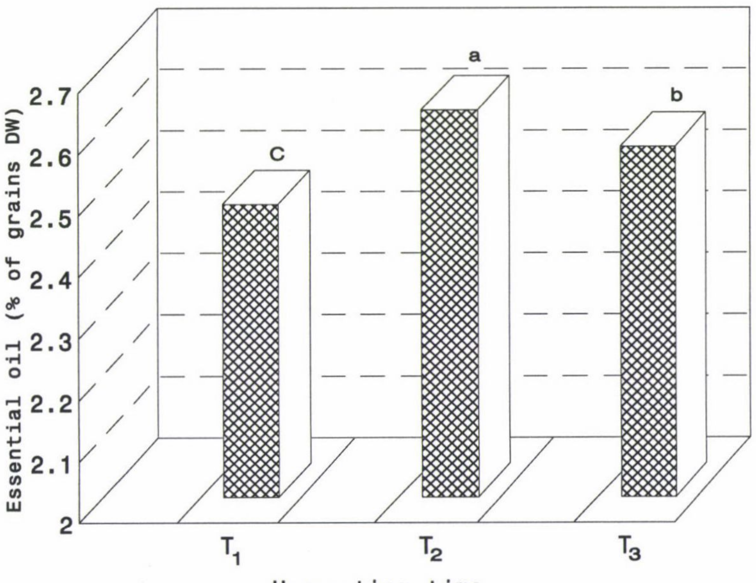

Fig. 4. Effect of harvesting time on the essential oil content of anise. T_1 = Milky stage, T_2 = Waxy stage and T_3 = Ripening stage

Discussion

Plant growth, dry matter and total essential oil production were decreased by diminishing the available water to below 80% (Table 1). These results are supported by earlier reports on anise (Abdel-Kawy et al., 1981; Hornok, 1992; Randhawa et al., 1992) and wheat (Van den Boogaard, 1995).

At low soil water availability, relatively more biomass was allocated to the roots than to the shoot (Table 1). Reduced development of leaf area may conserve water and prevent the adverse effect of water shortage on the plant shoot. Under field conditions, a higher leaf area will reduce evaporation from the soil surface and hence increase the amount of water that can be used for plant growth (Van den Boogaard, 1995).

The results of the field experiment indicated that anise growth was decreased by delayed sowing date and by water limitation at the stem elongation and umbel appearance stages (Tables 1 and 2). It should be noted that the effect of sowing date was less on the grain yield than on the growth of anise. This is in conformity with Fazekas et al. (1981) and Maheshwari et al. (1989).

The average grain yield ranged from 457.2 to 894.3 kg ha^{-1} in the field. Water deficit during stem elongation (SE) and umbel appearance (UA), when the plant grows fast, may reduce the anise yield (Abdel-Kawy et al., 1981; Hornok, 1990). Water limitation in the grain filling (GF) period had no great effect, but mean grain yield was decreased significantly by water deficit in the SE and GF stages (Fig. 2).

The mean essential oil yield was highest on plots sown earlier (April 4 to 16). The I_4 water deficit treatment had no appreciable effect on oil yield, but essential oil production was reduced significantly by the other water limitation treatments (Fig. 3). It seems that the increase in oil percentage under water deficit conditions resulted from a decrease in seed dry matter (Van den Boogaard, 1995). Therefore, in spite of the increased percentage of essential oil, the total amount of oil production was reduced in the water limitation treatments (Table 2 and Fig. 3).

It may be concluded that low available water (less than 80%) affects the growth, yield and essential oil production of anise. The results also indicate that for successful grain and oil production, this plant must be sown early in the spring (April 4 to 16) in Tabriz. Water deficit in the SE and UA periods may reduce the growth, yield and oil production of anise appreciably.

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WATER DEFICIENCY RESISTANCE STUDY ON SOYA AND BEAN CULTIVARS

E. NEMESKÉRI

AGRONA LTD., DEBRECEN, HUNGARY

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In a three-year model experiment the water deficiency resistance and the quality of yield were investigated in the case of soybean and bean cultivars of determined growth. The plants were grown under irrigated and non-irrigated conditions, modelling the conditions of drought. The soybean cultivars used a considerably higher amount of water before reaching the green pod maturity stage than the bean cultivars. Their water use under water stress decreased considerably (46%), in contrast with beans, where this decrease was only 18–21%. This correlates with the vegetation period of the varieties, their water use in certain vegetation periods and the development of their roots. The roots of beans determine the size of the yield and that of water circulation but only affects the formation of pods in a few varieties. The roots of soybean cultivars affect the size of yield and the quality of seeds as well. This is especially important in organic matter circulation under water-deficient conditions. If the roots are small, the dry matter and oil contents increase more intensively in the seed, but this has no effect on the protein content of the seeds. The small root mass and small specific leaf area (SLA) of the early soybean cultivar McCall and the Hungold variety of beans decreased yield losses under water deficiency conditions, in contrast with the mid-late maturing soybean variety Evans, where this was not experienced.

Key words: water stress, tolerance, quality, bean, soybean

Introduction

Climatic changes, leading to the lack of groundwater and frequent atmospheric droughts, urge researchers to increase the climatic resistance of legumes known to have high water requirements.

In the case of poor water stress, high atmospheric CO_2 levels cause partial stomatal closure and improve the water status of the plants, so that the growth of the leaves is enhanced. At high temperatures, the tolerance of plant varieties can be increased by the reduction of photorespiration and by increasing the water utilization efficiency of C3 type cultivars (Hsiao, 1994). Three genes are responsible for the development of heat-induced drought tolerance and affect the number of pods/plant in green beans (Bou et al., 1982). According to other authors (Parker, 1968; Velich, 1992), the development of drought tolerance is due to several simultaneous plant factors, which must be taken into consideration in plant breeding programme. In plants, the root:stem ratio, the stomatal size, cuticular resistance, atmospheric temperature and vapour conditions affect water loss from the leaves. In the case of soybean cultivars, in late vegetative or early reproductive development periods, without irrigation, the proportion of roots was found to increase and that of the stem to decrease. In the early stages of vegetative development (about the first 6 nodes), drought stress does not affect the development of the roots and stem (Hoogenboom et al., 1986) as the efficiency of water use increases (Neyshabouri, 1982). When investigating the effects of severe water deficiency in French beans, Nemeskéri (1990) found that tolerant types needed the largest amount of water during vegetative growth, resistant types during flowering, and susceptible types in the period of pod formation and development. Under dry conditions caused by the water deficiency of the soil, the root nodules of white beans aged, so their nitrogen-binding activity decreased compared to soybean cultivars, where this was not perceived (Smith et al., 1988).

As a result of water stress the development of plant leaves decreases in the initial period of growth, at a rate correlating with their structures. In several species (soybean, rice) Wallace et al. (1972) found that the photosynthetic activity of thicker leaves, especially if the leaf consisted of many minute cells, was greater than that of thinner leaves. At lower leaf water potentials, soybean cultivars close their stomata more rapidly, their water uptake from the soil is quicker and the efficiency of water utilization is lower than that of *Vigna* varieties (Lawn, 1982). Depending on the water deficiency tolerance of green bean cultivars, not only does the quantity of yield decrease, but its quality deteriorates as well. As a result of water deficiency, the protein content in the yield of tolerant types slightly increased, whereas the sugar content decreased compared to varieties grown under irrigated conditions (Nemeskéri, 1990). The raw fibre content of the green pod exceeded 11% in the case of varieties sensitive to water stress.

In this study the water utilization efficiency and water stress resistance were compared in French beans with high water requirements and in soybean cultivars. The correlation between the water stress resistance and the yield quality of the varieties was investigated in order to develop tolerant genotypes.

Materials and methods

In an experiment modelling three years of water deficiency the water stress resistance of bean and soybean cultivars of determined growth was investigated. The bean cultivars investigated were Hungold, Maxidor and Lada; the soybean cultivars were the early maturing McCall and the mid-late maturing Evans. Soil samples identical to those in field experiments were placed in large (28 cm × 28 cm) pots. Two seeds of each variety were sown in the pots. In the irrigated and non-irrigated (water-deficient) treatments, four pots arranged in four random replications represented each variety. Water portioning and weight measurements were performed every three days, both in the irrigated (I) and non-irrigated (NI) treatments. In the case of (I) the water dose was equal to field capacity (Vk=70%), while in (NI) half this quantity was used. The soybean and bean experiments were performed under identical experimental conditions and the data were evaluated with the same statistical methods.

The total water use was determined for unit plant yield (specific water use coefficient) and the quantity of water used in certain periods of plant development under irrigated and non-irrigated conditions was recorded.

In each irrigated and non-irrigated treatment the mass of certain plant parts was measured. The leaf area was measured, and the leaf area index (LAI) was determined to calculate the specific leaf area (SLA) related to the fresh mass of leaf area. Comparative analysis was performed up to the pod maturity stage of the soybean and bean cultivars.

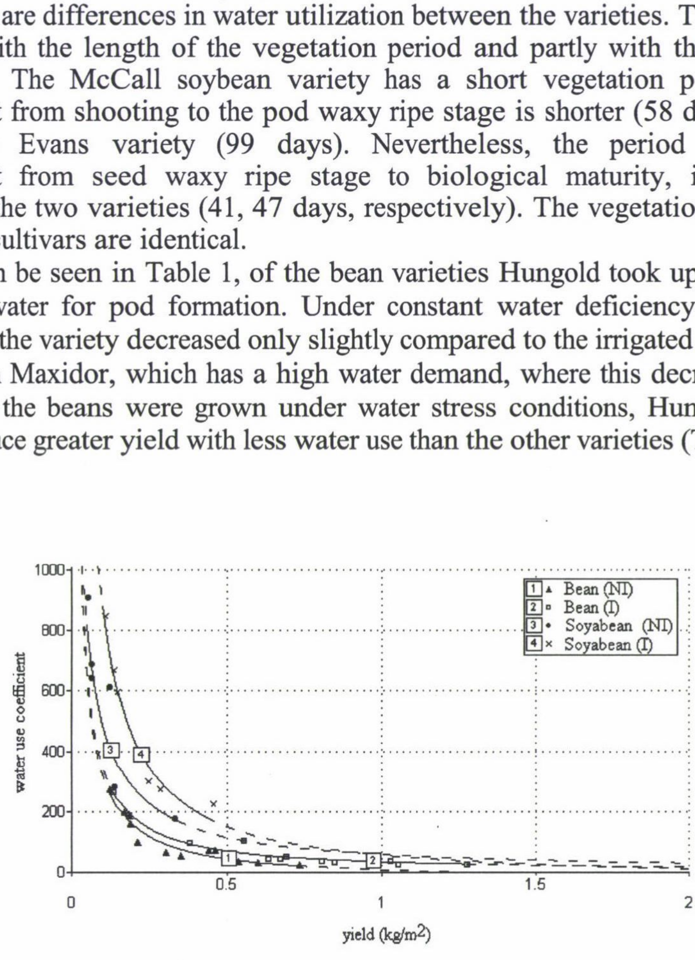
Trends in water use were investigated in the growing periods of the two plants. Regression analysis was performed to identify the dominant factors affecting plant yield and quality.

Results

In the soybean and bean cultivars the correlation between water use and yield was analysed up to the pod stage under irrigated and non-irrigated conditions. As shown in Figure 1, soybeans used more water in the green pod maturity stage than beans, but under water stress they could only take up water efficiently for a pod yield of 0.3 kg/m^2. For beans, the water utilization also decreased under water stress, but a pod yield of 0.5 kg/m^2 could be obtained. The significant correlation between water use and yield (r=0.96) shows the efficacy of water utilization (Fig. 1).

There are differences in water utilization between the varieties. This partly correlates with the length of the vegetation period and partly with the genetic background. The McCall soybean variety has a short vegetation period; its development from shooting to the pod waxy ripe stage is shorter (58 days) than that of the Evans variety (99 days). Nevertheless, the period of seed development from seed waxy ripe stage to biological maturity, is almost identical in the two varieties (41, 47 days, respectively). The vegetation periods of the bean cultivars are identical.

As can be seen in Table 1, of the bean varieties Hungold took up the least amount of water for pod formation. Under constant water deficiency (NI) the water use of the variety decreased only slightly compared to the irrigated plants, in contrast with Maxidor, which has a high water demand, where this decrease was 21%. When the beans were grown under water stress conditions, Hungold was able to produce greater yield with less water use than the other varieties (Table 1).



1 n=12 r=0.9656 p=0.1% y'=-27.622109+35.020494*1/X
2 n=12 r=0.9813 p=0.1% y'=-1.360269+35.032811*1/X
3 n=6 r=0.9550 p=1% y'=1/(0.000352+0.016915X)
4 n=6 r=0.9893 p=0.1% y'=-36.255087+92.251323*1/X

Fig. 1. Relationship between water use coefficient and pod yield in bean and soybean

Under properly irrigated conditions the maximum water use of the soybean cultivars was higher than that of bean cultivars and they suffered more from permanent water deficiency. Their water use decreased to a greater degree (46%) up to the pod formation stage than that of bean cultivars, for which the rate of decrease was about 18–21% (Tables 1 and 2). Water utilization may differ in the various development periods of the varieties. The early maturing McCall variety uses most of its water uptake (44.8–44.3%) for pod formation, irrespective of the water supply. The mid-late maturing Evans variety uses the majority of its available water supply (39.6%) during the flowering period, but takes up a considerable amount of water in the period of seed development as well (21.6%). A similar distribution ratio was observed under permanent water deficiency (NI), where 34.7% of the total water amount was used for flowering, and about 26% for seed development (Table 2).

Table 1

Water utilization in various development stages of beans under irrigated [I] and water stress [NI] conditions

Development stages	Maxidor	Maxidor	Hungold	Hungold	Lada	Lada
	[I]	[NI]	[I]	[NI]	[I]	[NI]
Shooting	3.55	4.09	3.65	3.65	3.58	4.06
Growth	10.02	7.95	9.84	8.45	9.97	8.60
Flowering	9.88	8.68	9.56	8.58	9.38	9.24
Pod lengthening	9.30	5.32	6.93	3.87	8.60	4.51
Total water use [litre]	32.75	26.03	29.98	24.83	31.53	26.40
Pod yield kg/m^2	0.85	0.32	0.81	0.46	0.73	0.39

Table 2

Water utilization in various development stages of soybeans under irrigated [I] and water stress [NI] conditions

Development stages	McCall	McCall	Evans	Evans
	[I]	[NI]	[I]	[NI]
Growth	11.46	8.62	16.48	11.63
Flowering	11.44	5.13	31.82	15.87
Pod lengthening	33.69	17.66	14.67	6.29
Seed development	18.62	9.40	17.32	11.92
Water used until pod lengthening [litre]	56.59	31.40	62.97	33.79
Total water use [litre]	75.21	40.81	80.29	45.71
Pod yield kg/m^2	0.51	0.24	0.30	0.14
Seed yield kg/m^2	0.11	0.06	0.45	0.33

Plant analysis was performed to investigate the reasons for the differences in water utilization between the various plants. The biological difference between the two species is demonstrated by the fact that the root mass of soybean is considerably bigger than that of beans, while its stem mass, leaf mass and specific leaf area are also slightly bigger (Table 3). Although the roots of soybeans are about three times bigger than those of beans, both soybean and bean cultivars react to water deficiency stress with a considerable mass loss of 42–45%. Similarly to root mass, the stem mass decreased as well, but to a greater extent (49–55%) in the case of soybeans. Water stress caused less leaf mass decrease in soybeans than in the bean cultivars.

The bean cultivars reacted differently as regards the development of leaf mass, leaf area and leaf number, so an attempt was made to demonstrate the differences by defining the specific leaf area (SLA) (Table 3).

Under water stress, the bean varieties and McCall soybeans, which belong to the same ripening group (90–100 days), showed a moderate decrease in pod number and a considerable decrease in pod weight compared to irrigated plants (Table 3). In the case of Evans, which has a longer vegetation period, water stress considerably inhibited pod and seed development. The latter was evaluated in the waxy-ripe stage.

Investigations were made on the correlation of the major factors in water stress resistance, which could explain the water stress reactions shown by both plant species.

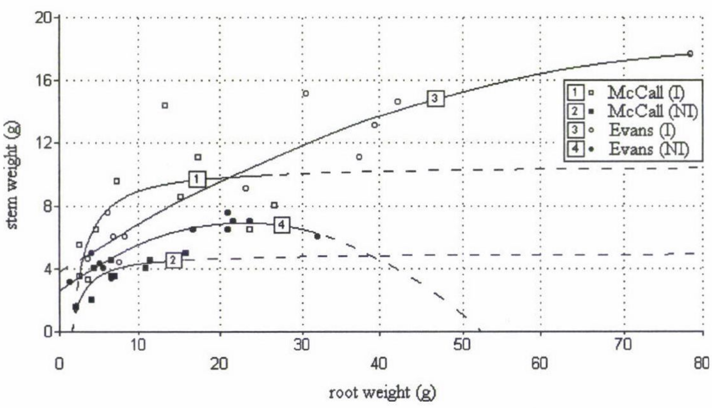
The correlations between the yield-determining biological factors showed that the development of the roots is dominant in providing stem and pod mass in the case of both soybean and bean cultivars. The roots of beans provide 50% of the yield but only affect pod formation in a few varieties.

The roots of certain bean cultivars with great water demand, e.g. Maxidor, react to water deficiency very sensitively, so the roots cannot play an important part in yield stability. Yield decrease is caused by extensive root destruction. According to Nemeskéri (1990), cultivars with small root mass, e.g. Hungold, are important in providing pod weight in the case of permanent water deficiency. The medium correlation coefficient (r=0.52%) between root weight and pod weight shows that its water utilization is better in drought than that of Maxidor. With the Hungold and Lada varieties, besides the small root mass, the area of assimilation is of key importance in providing the water circulation of the yield. In the case of the Lada cultivar, there is a significant correlation between leaf area and pod weight (r=0.66) and between leaf weight and pod weight (r= 0.58), respectively.

Figures 2 and 3 demonstrate the role of the root mass of soybean cultivars in plant growth and the quantity of yield. Under both irrigated and water deficient conditions, there is a close correlation between the root weight and stem weight in soybean cultivars. Differences between varieties can be observed, as the root weight of the McCall cultivar shows moderate growth under irrigated and non-irrigated conditions as well. As the regression curve in Figure 2 shows, the development of the roots, after reaching a mass of 15 g, inhibits the growth of stem mass. The even root development of the Evans variety becomes slow under water deficient conditions; after reaching 20–22 g, the stem mass reaches a maximum value (6.5–7 g), followed by root deterioration and stem mass loss. Our findings showed a close correlation between root mass and pod mass under both irrigated (r=0.84–0.9) and non-irrigated (r=0.84–0.87) conditions (Fig. 3). This means that the development of pod mass and its water circulation, as in beans, is primarily affected by the development of the roots. As the regression curve shows, under irrigated conditions at a mass of 22 g the roots of the McCall cultivar provide the maximum pod weight (23 g). Under water deficient circumstances, less root mass (15 g) is able to produce about 21.7% less yield (about 17–18 g of pods).

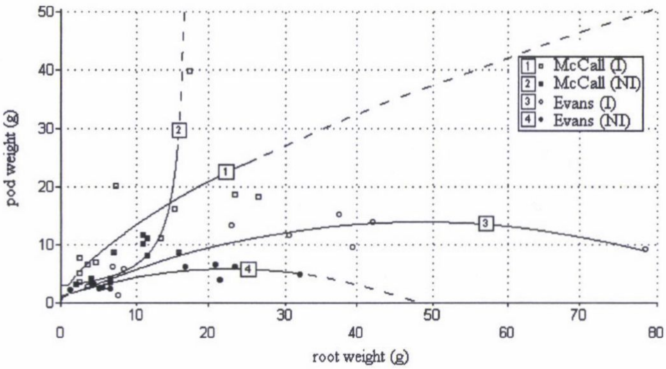
Under irrigated conditions, a root mass of 22 g in the Evans cultivar, considering the volume of the stem mass (Fig. 2), can produce 10 g of pod yield. In the case of non-irrigated conditions, even if the root mass reaches a volume of 30 g, there is a considerable decrease both in the stem and pod masses.

With soybean cultivars, the quality components of the leaves and seeds were investigated, including the role of the roots in irrigated and non-irrigated plants.



1	n=11	r=-0.7430	p=1%	y'=10.479227-16.622681*1/X
2	n=11	r=-0.8567	p=0.1%	y'=4.966319-7.351113*1/X
3	n=11	r=0.9433	p=0.1%	$y'=3.767289+0.317799X-0.001828X^2$
4	n=11	r=0.9275	p=0.1%	$y'=2.531238+0.375481X-0.008127X^2$

Fig. 2. Relationship between root weight and stem weight in soybeans under irrigated [I] and water stress [NI] conditions



1 n=11 r= 0.8389 p=1% $y'=3.006213X^{+0.641835}$
2 n=11 r=-0.8362 p=1% $y'=1/(0.355448-0.019938X)$
3 n=11 r= 0.8993 p=1% $y'=0.735585+0.53719X-0.005547X^2$
4 n=11 r= 0.8685 p=1% $y'=1.041364+0.406361X-0.008908X^2$

Fig. 3. Effect of root system on pod weight in soybean under irrigated [I] and water stress [NI] conditions

Correlation analysis was extended to identify the correlation between root mass and seed dry matter, protein and oil content as well. The smaller roots of the McCall cultivar allow intensive dry matter intake under both water deficient and irrigated conditions. With the decrease in root mass, the seed dry matter content growth of the Evans cultivar is slower (Fig. 4). Under water deficient conditions, there is a significant, close correlation (r=0.78) between the root mass and oil content, where the seed oil content reaches a maximum value (23%) when the root mass is 15 g, after which it decreases. In the case of the McCall cultivar, there is only a medium correlation (r=0.52) between root mass and oil content under water deficient conditions.

Under irrigated conditions, the roots did not affect the oil content of the seeds in any of the cultivars.

Under water deficient conditions a close, significant correlation (r=0.706) between root mass and seed protein content could only be found in the Evans variety. There was no significant difference in the rate of incorporation of seed protein in the case of either irrigation or non-irrigation. In the McCall variety, the roots did not play a key role in the accumulation of seed protein.

According to the data in Table 4, under water deficient circumstances, the leaf protein content significantly decreased in the early stage of waxy ripeness (a), and there was also a slight decrease in seed protein content. At the time of physiological seed maturity (b) protein started to migrate from the leaves towards the yield, and the difference between irrigated and non-irrigated plants was evened out. In the case of irrigation there was a slight increase in the incorporation of seed protein but in water deficiency this process came to a halt. The trypsin inhibitor activity of the seed (TIU) increased during seed development. Under water deficient conditions, in accordance with earlier results (Nemeskéri, 1997), the TIU content was higher in water deficiency than in irrigation.

Table 3
Effect of water stress on different parts of bean (Maxidor. Hungold and Lada) and soybean (McCall and Evans) plants

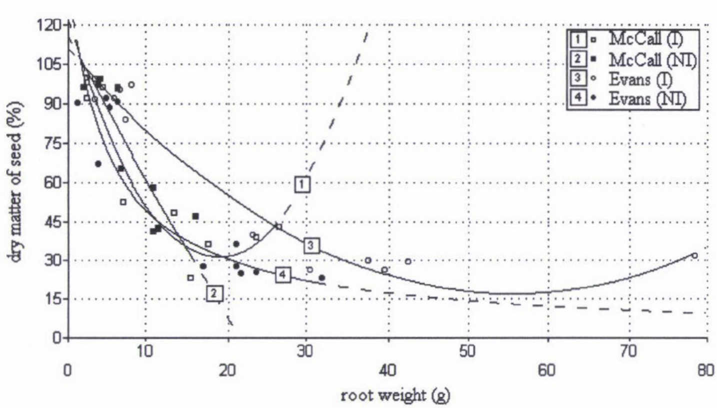
Cultivar	Treatment	Root weight (g)	Diff. (%)	Stem weight (g)	Diff. (%)	Leaf weight (g)	Diff. (%)	SLA	Diff. (%)	Pod weight (g)	Diff. (%)	Pod number	Diff. (%)
Maxidor	I	4.84		5.08		6.51		0.49		13.9		7.54	
	NI	2.79	–42.36	2.82	–44.49	4.17	–35.95	0.39	–20.41	8.0	–42.45	4.55	–39.66
Hungold	I	3.16		3.18		3.96		0.50		13.6		6.58	
	NI	1.83	–42.09	1.97	–38.05	2.26	–42.93	0.55	+10.0	8.8	–35.29	5.24	–20.36
Lada	I	3.54		4.26		5.99		0.39		11.1		4.92	
	NI	2.40	–32.20	2.67	–37.33	3.57	–40.41	0.44	–12.82	7.2	–35.14	3.81	–22.56
McCall	I	17.29		9.63		6.03		0.74		20.5		13.67	
	NI	11.33	–34.64	4.25	–55.87	4.38	–27.36	0.56	–24.32	9.58	–53.27	8.33	–39.06
Evans	I	41.88		13.34		7.29		0.80		12.0		12.25	
	NI	22.67	–45.87	6.75	–49.41	7.38	+1.23	0.58	–27.50	5.59	–53.42	6.08	–50.37

The data refer to one plant. Diff.: % difference between irrigated [I] and hardly irrigated [NI] plants; SLA: specific leaf area

Table 4
Effect of water stress on seed quality of soybean

Treatment	Plant organ	Dry matter (%)			Protein (%)			Oil (%)			TIU		
		a	b	c	a	b	c	a	b	c	a	b	c
[I]	Leaf	43.69	55.54	–	11.5	9.09	–	3.89	3.14	–	–		
[NI]	Leaf	35.34	39.21	–	7.74**	8.89	–	3.75	3.57	–	–		
Difference+		–8.35	–16.33		–3.76	–0.2		–0.14	0.42				
$LSD_{5\%}$		ns.	23.1		3.7	ns.		ns.	ns.				
[I]	Seed	33.04	36.94	93.34	25.68	26.99	28.84	23.93	19.35	21.51	17.35	21.78	36.17
[NI]	Seed	34.64	41.13	90.27	24.38*	26.02	26.99	24.03	20.06	19.71	24.69	31.74	33.29
Difference+		1.6	4.19	3.07	–1.3	–0.97	–1.85	0.1	0.71	–1.8	7.34	9.96	–2.88
$LSD_{5\%}$		ns.	ns.	ns.	1.58	ns.	–	ns.	ns.	ns.			
$LSD_{10\%}$					1.15		3.37						

+between [I] and [NI]; [I] irrigated treatment, [NI] hardly irrigated treatment = water stress. Trypsin inhibitor amounts are expressed in trypsin inhibition units [TIU] per mg dry matter, a =early stage of waxy ripeness, b =physiological seed ripening, c =dry seed. *significant differences, ns.=non-significant



[1] n=11 r=0.9626 p=0.1% $y'=120.678888-9.451982X+0.248259X^2$
[2] n=11 r=-0.8759 p=0.1% $y'=114.291981-5.324074X$
[3] n=11 r=0.9760 p=0.1% $y'=109.705918-3.360872X+0.030257X^2$
[4] n=11 r=0.9439 p=0.1% $y'=1/(0.007456+0.001292X)$

Fig. 4. Effect of root system on dry matter of seed in soybean under irrigated [I] and water stress [NI] conditions

Discussion

The root development of the varieties and the volume of the assimilation area are of key importance in the water and nutrient circulation of plants and thus in the development of yield. The different reactions of the cultivars suggest that the water deficiency resistance of the varieties could be enhanced even among leguminous varieties with high water demand. Genotypes with good water stress resistance can be improved by the application of selective methods based on the correlation of key biological factors in yield production (Nemeskéri, 1990; Velich, 1992). Soybean cultivars use more water than beans, but the resistance of soybean to permanent water deficiency is poorer. This correlates with the vegetation period of the cultivars, their water use in certain periods of development and the development of their roots. Early maturing soybean cultivars, similarly to beans, use most of the water for pod formation, whereas the mid-late variety Evans uses it for flowering.

From among the beans grown under water deficient circumstances, Hungold was able to form greater yield using less water, due to its small roots and advantageous root/stem ratio.

Independently of their vegetation period, the roots of soybean cultivars determine the volume of the pod yield (r=0.83–0.86) to a greater extent than those of beans, where the root mass plays a smaller role in the process (r=0.52–0.61). In water deficiency, not only the roots, but also the specific leaf area is a key factor in the development of the bean yield. Under water stress conditions tolerant plants compensate for the loss of leaf mass with the formation of numerous, thicker leaves. The photosynthetic activity of smaller, thicker leaves is favourable, so plants do not suffer from water deficiency (Wallace et al., 1982; Lin and Markhart, 1996). The present results indicate that the specific leaf area (SLA) is cultivar-specific and can thus be applied in beans for selection to increase water stress resistance. If the SLA value decreases in water deficiency less than in irrigated plants, it means that the cultivar is resistant to water stress.

Among the soybean cultivars, the early McCall shows a significant correlation (r=0.92) between leaf area and pod weight and a correlation of (r=0.84) between the mass of seeds in the waxy-ripe stage and the leaf area under water deficient circumstances. This means that, similarly to beans, the roots and leaf area of the McCall cultivar both affect the formation of pod yield. Its smaller roots promote more intensive incorporation in dry matter and oil content than in the case of the mid-late Evans cultivar. In the latter, neither the root/stem ratio nor the formation of the leaf area had an advantageous effect on pod yield under water-deficient conditions.

The small root mass and lower specific leaf area (SLA) of the early maturing McCall soybean cultivar helps to prevent yield loss under water deficient circumstances. On the basis of our findings, water stress resistance could be enhanced by selecting for root mass. In French beans, besides root mass selection, SLA selection should also be performed for the selection of genotypes resistant to water deficiency.

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Short communication

MUTANTS OBTAINED BY CHRONIC GAMMA IRRADIATION FROM A CARPATHIAN-UKRAINIAN LOCAL SOYBEAN [*Glycine max* (L.) Merrill] VARIETY: I. M_3 AND M_4 GENERATIONS

M. HAJÓS-NOVÁK[1] and F. KŐRÖSI[2]

[1]DEPARTMENT OF GENETICS AND PLANT BREEDING, FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, SZENT ISTVÁN UNIVERSITY, GÖDÖLLŐ, HUNGARY
[2] INSTITUTE OF ENVIRONMENTAL AND LAND MANAGEMENT, SZENT ISTVÁN UNIVERSITY, GÖDÖLLŐ, HUNGARY

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Mutant soybean germplasm was developed from a Carpathian-Ukrainian local variety, using 100–300 Gy chronic gamma irradiation to obtain lines with improved oil and/or protein content. The mutant germplasm was developed by the pedigree method. Selection for high oil and protein content started in the M_3 generation. Plants with 24.1 and 23.6% oil content in the seeds were detected in the M_4 generation. There were negative, moderate ($r = -0.4$) and significant ($P<0.1$ and $P<0.01$) correlations between the oil content and the 1000-seed weight in both the M_3 and M_4 generations. The fatty acid composition in the seeds of plants with high oil content was favourable. It is suggested that selection for oil content in the seeds should be started in the M_4 generation. Due to the limited genetic variation for protein content no mutant genotypes with higher protein content than that of the control could be identified.

Key words: soybean, chronical gamma irradiation, oil and protein content, mutants

Introduction

Soybeans provide over half of the world's supply of vegetable protein and about one-third of the oil (Fehr, 1989). They are processed into defatted meal flakes and crude oil. Because of frequent fluctuations in the relative market value of protein and oil, it is essential to improve both the meal and oil fraction of soybeans.

Mutant populations produced by irradiation provide a good source for breeders to develop soybean cultivars with high oil and intermediate protein and/or high protein and intermediate oil content. During the last 30 years about 30 soybean cultivars have been produced by X-rays, thermal neutron and gamma irradiation in different countries, mainly in China (Wang and Licheng, 1995). These released cultivars showed improvement in maturity, yield, resistance to lodging, tolerance to pod shattering, first pod height, resistance to cyst nematode, oil and protein content. In Turkey, a mutant variety, TAEK A-3, with high oil (25.5%) and high protein content (39.2%) was registered (Sagel et al., 1995). Thus, the objective of this present study was to obtain soybean lines with higher oil and/or protein content and acceptable agronomic characteristics.

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Materials and methods

Samples of 500 seeds each of a Carpathian-Ukrainian local variety were sown in the gamma field of the Department of Genetics and Plant Breeding, Gödöllő, and exposed to gamma rays from a ^{60}Co source at a 100–300 Gy dose during the whole vegetation period. All non-lodging M_1 plants were harvested separately and seeds from these single plants were sown in individual plots. Pedigree selection was started in the M_2 generation. Selection of single plants was carried out for earliness, first pod height, number of pods, 1000-seed weight, yield per plant, harvest index, lodging and shattering. Fifty seeds from each of 20 to 30 M_3 plants per dose were grown in progeny rows, and selected in the M_4 generation for the agronomic characteristics mentioned above.

Oil content and fatty acid composition were analysed in M_3 and M_4 seeds using nuclear magnetic resonance (NMR) and gas liquid chromatography. The crude protein content was measured in the M_4 generation by an automatic Kjeldahl method.

For data evaluation, i.e. test of normal distribution, analysis of variance and regression analysis (Mendenhall, 1987) were applied using the Statgraphic version 4.0. Furthermore, a function index was calculated by dividing the density function and the survival function, equal to 1- the cumulative distribution function, to predict the genetic variability for the oil content.

Results

Oil content (%) and 1000-seed weight (g) in the M_3 generation

The oil content of the seeds in the M_3 generation ranged from 17.6 to 23.1%, with an average of 19.3%. The M_3 mutant lines showed a slight (±3%) variability in oil content. The normal distribution of the oil content data was proved at 10% by the χ^2 test ($\chi^2 = 6.26$).

As one of the most important yield components, the 1000-seed weight was also evaluated in the M_3 generation; it ranged from 160.0 to 275.7 g. On the basis of the χ^2 values this character showed significant normal distribution at the 0.01% probability level.

The correlation between oil percentage and 1000-seed weight was negative, moderate ($r = -0.4$) and significant ($P<0.1$). It can be predicted, using linear regression analysis, that a 10.0 g increase in 1000-seed weight may result in a decrease in oil content of about 0.16%.

Oil content (%) and 1000-seed weight (g) in the M_4 generation

In the M_4 generation the oil content ranged between 19.8 and 24.1% with an average of 21.8%. The highest oil contents of 24.1 and 23.6% were achieved by the variants 150 Gy and 100 Gy which were 4.3 and 3.9% higher as compared to the control. The function index predicts up to 28% oil content in the irradiated Carpathian-Ukrainian germplasm (Fig. 1).

Comparing the oil content of the seeds of the studied generations, it was found to be 1.2–1.5% higher in the M_4 than in the M_3 generation. Gamma irradiation had no effect on the 1000-seed weight at most of the applied doses, except for 150 Gy, which decreased it from 175.0 to 148.1 g. High variance values in the progeny of different doses indicate the presence of considerable genetic variability. This characteristic is strongly influenced by environment; thus, detailed tests must be carried out in order to identify the relative portion of genetic and environmental variance.

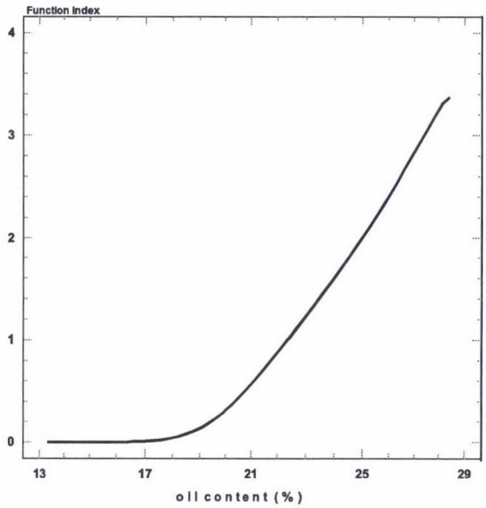

Fig. 1. Function index for the oil content distribution of the mutant Carpathian-Ukrainian soybean germplasm in the M_4 generation

Regression analysis of the oil content and 1000-seed weight revealed the expected negative correlation between the two characteristics. The correlation coefficient was similar than in the previous generation ($r = -0.4$, $P<0.01$). In the M_4 an increase in 1000-seed weight of 10.0 g resulted in a decrease in oil content of 0.17%.

Fatty acid composition in the M_3 and M_4 generations

Soybean oil contains more than 50% polyunsaturated fatty acids and about 15% saturated fatty acids, most of which is palmitic acid ($C_{16:0}$). The quality of soybean oil is also affected by its linolenic acid ($C_{18:3}$) content. A reduction in the percentage of linolenic acid should enhance the quality, i.e. the stability of the oil. In the M_3 generation, only seeds with favourable oil content were analysed for fatty acid composition. The linoleic acid ($C_{18:2}$) content in the seed oil ranged from 45.4 to 55.0% and the linolenic acid portion from 5.5 to 8.6%.

In the M_4 generation seeds exposed to 150 Gy gamma radiation achieved the lowest linolenic acid content (8.3%). These seeds had 24.1% crude oil on a dry matter basis. The palmitic acid ($C_{16:0}$) content of the 150 Gy treatment was only 9.3%, and the corresponding linoleic content was 54.5% (Table 1).

Protein content (%) in the M_4 generation

M_4 plants with high and low oil content in the seeds were checked for their protein content. The crude protein content of the M_4 seeds ranged between 27.4 and 38.7% with an average of 35.0%, i.e. chronic gamma irradiation did not affect the protein content. Due to the negative correlation between the oil and the protein content, seeds with an oil content of 24.1% had only 27.4% crude protein.

Table 1
Oil content (%) and fatty acid composition (%) of M_4 mutant Carpathian-Ukrainian soybean progeny rows

Treatment	Oil content (%)	Palmitic acid (%) $C_{16:0}$	Stearic acid (%) $C_{18:0}$	Oleic acid (%) $C_{18:1}$	Linoleic acid (%) $C_{18:2}$	Linolenic acid (%) $C_{18:3}$
Control	19.8±0.32	9.7	5.2	22.5	53.0	8.3
100 Gy	23.6±0.95	9.4	5.3	22.1	51.6	8.7
150 Gy	24.1±1.10	9.3	5.1	20.9	54.5	8.3
200 Gy	21.5±0.71	9.3	4.8	19.7	55.6	8.8
300 Gy	20.0±0.82	10.8	4.5	21.4	51.4	10.0

Discussion

The results presented demonstrate that mutation induction in soybean can be successfully used to improve seed characteristics. Soybean mutants with high oil (24.1%) and favourable fatty acid composition were selected from a Carpathian-Ukrainian local variety. Using radiation techniques, similar improvements in the oil and protein contents of soybean were achieved by Williams and Hanway (1961), Sagel et al. (1995), Wang et al. (1989) and Wang and Licheng (1995).

By growing soybeans with higher oil contents (24%), even with a loss of crop yield, farmers could theoretically achieve an oil yield of about 460 kg/ha.

Seed quality should be checked in the M_5 generation in order to reveal whether the lines are homozygous for oil content. Progeny rows for characteristics with agronomic importance, like earliness, first pod height, number of pods, 1000-seed weight, yield per plant, harvest index, lodging and shattering, should also be studied.

Acknowledgements

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Short communication

EXPERIMENTAL IMPROVEMENT AND EVALUATION OF VERTICAL INTENSIVE CROWN FORMS

T. BRUNNER[1], E. PÁLDI[2], L. JUHÁSZ[3], F. TÓTH[3] and J. IVÁNCSICS[4]

[1]JURED COMPANY, KISKŐRÖS
[2]AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES, MARTONVÁSÁR, HUNGARY
[3]HEJŐMENTI AGRICULTURAL COMPANY, MEZŐCSÁT, HUNGARY
[4]DEPARTMENT OF HORTICULTURE, UNIVERSITY OF WEST HUNGARY, MOSONMAGYARÓVÁR, HUNGARY

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The physiologically-based pruning methods elaborated by the authors were found to increase intensity in various ways, including early fruiting, improved yield and quality, and a reduction in the height of the cropping area, allowing at least 80% of the fruit to be picked from the ground. Compared with the Lespinasse control, crowns with a valve-like central leader on a sectorial spindle gave a surplus yield of 8.5 t/ha/year over the average of 3 years, including an increase of 2.3 t/ha/year in the yield of extra quality and grade I fruit.

Key words: sweet cherry, valve-like central leader, vertical crown forms, Lespinasse, yield and quality

Introduction

The starting-point for the experiments was the vertical axis crown form (Fig. 1a) elaborated for apple trees by Lespinasse (1986), who considered this crown form, which requires a maximum growing space of 4×2 m, to be suitable principally for Golden Delicious, Smoothee, Jonathan, Idared and Gloster 79. In this crown form the axis dominates the fruit-bearing side branches, which are located directly on the vertical axis. The axis itself does not require pruning, while the side branches, which are located favourably for fruit formation, need to be thinned and rejuvenated from time to time. It is important to avoid both the over-weakening of the tree, and thus of the vertical axis, and an excess of growth vigour. In the first case the tree cannot develop a satisfactory cropping area, while in the second case the excessive vegetative growth may lead to the lower branches becoming bare.

Nevertheless, despite the weakening of the rootstock by the well-balanced form and density of the vertical axis, the rapid fruiting and the increase in the height of the cropping area soon necessitates the use of a tractor-drawn platform to facilitate pruning and harvesting. Increasing intensity while keeping the cropping area at a low height, thus eliminating the need for a platform during pruning and harvesting, would be an advantage not only in the case of apple trees but also in other fruit species.

Materials and methods

In both of two variants of the Lespinasse vertical axis, one having a false central leader on a sectorial axis and the other a valve-like central leader on a sectorial spindle, the spreading of the side branches was achieved in a similar manner with the aid of delayed sectorial double pruning and epinastic bending. However, these two crown forms differed from each other in the training of the central leader. In the first the bending of the side branches by pruning was combined with the training of a false leader, in order to avoid excessive upward growth (Fig. 1b), while in the second the central leader was allowed to grow above the top storey and the branches growing on it were cut back to one or two internodes. This long axis, which can be pruned with long-handled pruning shears or from a ladder (Fig. 1c), needs to be cut back every three or four years when its growth becomes excessive. This valve-like axis is able to reduce the growth vigour by syphoning off the superfluous vegetative energy. No fruit should be allowed to grow on this central leader, but should be removed as buds in spring, since the weight of the fruit would cause the vertical axis to bend, thus reducing its growth regulating effect. The presence of this central leader also prevents any of the end shoots in the upper storey from trying to take its place due to physiological tip regeneration. These upper side branches can also be weakened by applying delayed sectorial double pruning, which has an intensely weakening and yield-enhancing effect, at an earlier date, while delaying the application of this technique on the lower branches. In this way the lower branches will be longer and the upper branches shorter, so that the whole of the cropping area will be better illuminated.

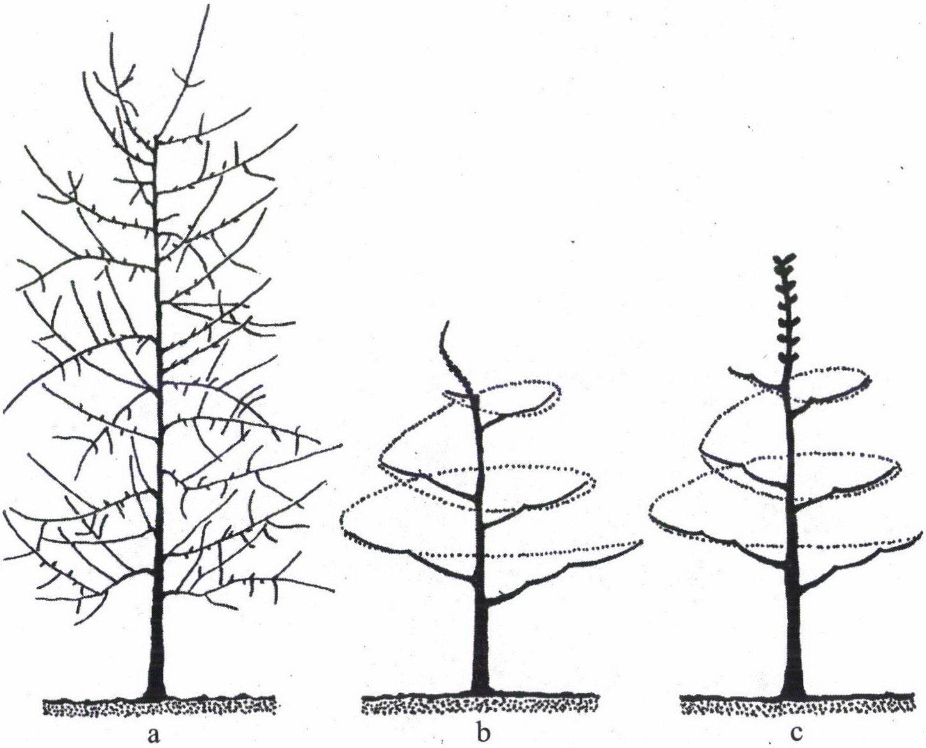

Fig. 1. Crown forms studied in the experiments. a. Lespinasse vertical axis. b. False central leader on a sectorial axis. c. Valve-like central leader on a sectorial spindle.

Results and discussion

For the crown forms described above, sectorial double pruning was aimed at eliminating apical dominance on the lower side of slanting branches. This necessarily leads to the growth of shoots closer to the horizontal. This bending effect is enhanced by the end shoot formed from the upper bud, which is not removed until the end of the vegetation period. This bending is continued by again pruning the slanting branches thus obtained to an upper bud, without the need for tying or a support system. This sectorial double pruning reduces the manual labour requirements of bending to a third or a sixth. It is referred to as "sectorial" because it makes conscious use of the one-sided material transport disturbance induced by pruning. The term "double pruning" is used because pruning to an upper bud is followed by the removal of the terminal branch from above the "bent" branch. Delayed sectorial double pruning differs from this only in that the terminal branch is not removed until the second year, thus prolonging the dominance of the terminal shoot arising from the upper bud. The slanting branch below it is left to grow, and its close to horizontal position results in a change in the auxin/gibberellic acid/cytokinin ratio, thus doubling or tripling the density of fruiting sections. Compared with the control, the valve-like central leader crown form gave a yield surplus of 8.5 t/ha over the average of three years, including an increase of 2.3 t/ha in the yield of extra quality and grade I fruit (Table 1). This was achieved with a reduction in the manual labour requirements.

In 1997 the total yield obtained on the valve-like central leader crown form was twice that of the control, while the yield of extra quality fruit was tripled (Table 2).

Table 1

Productivity (yield) of three vertical crown forms on the average of three years (1993, 1995, 1996) in the orchard of the Jured Company.

Treatment	Total		Extra qual.		Grade I		Grade II		Ungraded	
	t/ha	rel.%	t/ha	rel.%	t/ha	rel.%	t/ha	rel.%	t/ha	rel.%
Lespinasse vertical axis (control)	28.9	100	18.1	63	5.2	17	2.8	10	2.8	10
Relative %	100	–	100	–	100	–	100	–	100	–
False central leader on a sectorial axis	30.4	100	14.2	47	7.0	23	6.7	22	2.5	8
Relative %	105	–	78	–	135	–	239	–	89	–
Valve-like central leader on a sectorial spindle	37.4	100	16.7	45	8.9	24	9.3	25	2.5	6
Relative %	129	–	92	–	171	–	332	–	89	–
$LSD_{5\%}$	4.7	–	0.9	–	0.6	–	–	–	–	–
$LSD_{1\%}$	6.5	–	1.3	–	0.9	–	–	–	–	–
$LSD_{0.1\%}$	9.0	–	1.8	–	1.2	–	–	–	–	–

Note: Year of plantation: 1989; variety: Idared; rootstock: M4; spacing: 5×3 m; 666 trees/ha Minimal diameter (mm) and minimum skin colour (%): Extra quality: 70 mm and 50%; Grade I: 65 mm and 30%; Grade II: 65 mm and 15% (Compulsory EU standard – Rheinischer Landwirtschaft Verlag GmbH, Bonn, 1990)

Table 2
Productivity (yield) of two vertical crown forms in 1997 (8 years old) in the orchard of the Jured Company.

Treatment	Total		Extra qual.		Grade I		Grade II		Ungraded	
	t/ha	rel.%	t/ha	rel.%	t/ha	rel.%	t/ha	rel.%	t/ha	rel.%
Lespinasse vertical axis (control)	28.3	100	16.1	57	11.0	39	0.3	1	0.9	3
Relative %	100	–	100	–	100	–	100	–	–	–
False central leader on a sectorial axis	58.6	100	47.5	81	11.1	19	–	–	–	8
Relative %	207*	–	295*	–	101^{NS}	–	–	–	–	–

Note: Year of plantation: 1989; variety: Idared; rootstock: M4; spacing: 5×3 m; 666 trees/ha; 1) Standard deviation and the t-test were used in the statistical analysis. The values are the means of at least 6 measurements. NS = Non-significant; * = Significant at the $P \leq 0.01$ level. 2) For EU standard see note to Table 1.

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Short communication

EFFECT OF DATES AND RATES OF SOWING ON YIELD AND YIELD COMPONENTS OF NARBON VETCH UNDER SEMI-ARID CONDITIONS

A. M. TAWAHA and M. A. TURK

DEPARTMENT OF PLANT PRODUCTION, FACULTY OF AGRICULTURE, JORDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, IRBID, JORDAN

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Field experiments were conducted during the winter seasons of 1998–1999 and 1999–2000 at Houfa in northern Jordan, to study the effect of the date and rate of sowing on the yield and yield components of narbon vetch (*Vicia narbonensis* L.). Progressive delays in sowing beyond 1st January led to yield reductions of 11.1 and 17.9 at successive 15-day intervals. Plant height, pods stem^{-1}, stems m^{-2} and 1000-grain weight followed the same trend as the yield. Grain yield was not significantly affected by the sowing rate.

Key words: *Vicia narbonensis* L., sowing date, sowing rate, grain yield

Introduction

Narbon vetch (*Vicia narbonensis* L.) is a common forage legume in the rainfed, semi-arid system of the Mediterranean region. It is used for high quality hay production (Droushiotis, 1985) or for grazing after the beginning of the pod formation stage, or for grain and straw production whether grown in monoculture (Droushiotis, 1985) or in a mixture with cereals (Osman and Nersoyan, 1986). Narbon vetch is considered by Abd El Moneim (1992) to be one of the most attractive legume species for grain and straw production as feed resources in dry areas. Narbon vetch production under a particular set of environmental conditions is influenced by various agronomic factors. Among these, sowing rate and sowing date are the most important for production. Therefore, the present investigation aimed to examine the effect of sowing rate and sowing date and their interactions on the yield and yield components of narbon vetch under semi-arid conditions.

Materials and methods

A 2-year field study was carried out during 1998–99 and 1999–2000 at Houfa. The location has a Mediterranean climate with mild rainy winters and a dry hot summer. A fertilizer treatment of 30 kg ha^{-1} P_2O_5 was applied and mixed with the soil prior to planting. A split-plot arrangement in a randomized complete block design with three replications was used. Seeds of the locally planted narbon vetch cultivar (*Vicia narbonensis* L.) were sown on different dates (1st Jan., 15th Jan. and 2nd Feb.) in the main plots, with seeding rates (75 and 120 seed m^{-2}) in the sub-plots. The sub-plot area was 4.0 m^2 and consisted of 4 rows (2 outer rows as borders), 0.25 m apart and 4 m long. Guard strips of 0.5 m were left between the main plots and 1.5 m between the blocks.

The measured variables included seed yield (kg ha^{-1}), 1000 seed weight (g), pods stem^{-1}, stems m^{-2} and plant height (cm). The analysis of variance and LSD mean separations were performed using the MSTAT-C computer statistical program for RCBD with a split-plot arrangement according to the procedure outlined by Steel and Torrie (1980). Comparisons between means were made using the least significant difference test (LSD) at the 0.05 probability level.

Results and discussion

The grain yield of narbon vetch was influenced significantly by the date of sowing (Table 1). The maximum grain yield of 968.5 kg ha^{-1} was obtained by sowing narbon vetch on 1st January, which was found to be superior to the later dates of sowing, with progressive delays in sowing beyond 1st January leading to yield reductions of 11.1 and 17.9% at 15-day intervals. The seed yield of narbon vetch at different dates of sowing was significantly influenced by its attributes, i.e. plant height, pods stem^{-1}, stems m^{-2} and 1000-grain weight which followed the same trend as yield. The reduction in grain yield due to the delay in sowing can also be attributed to the shorter growth period at the disposal of late-sown crops, as the time taken by the crop to mature decreased with the delay in sowing.

The grain yields of the two seeding rates were not statistically different. The result found in this study is not consistent with Turk (1999) who found a significant effect of seeding rate on narbon vetch grain yield. This may be attributed to the diverse agroclimatic conditions. It is obvious from the above discussion that supplementing the traditional seed rate of 75 kg ha^{-1} does not contribute to a significant increase in the narbon vetch grain yield in the north of Jordan. It is concluded that the higher seeding rate causes higher inter-plant competition and results in poor individual plant vigour. In other words, the increased plant population reduces the branching of the individual plants. Therefore, the application of 75 kg seed/ha is recommended.

Table 1

Yield and yield components of narbon vetch (*Vicia narbonensis* L.) as influenced by seeding date and date of sowing

Treatments	Grain yield (kg ha^{-1})			Plant height (cm)			1000 grain weight (g)			Pods per stem			Stems/m^{-2}		
Grain rate	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Low	825.0	863.3	844.2	24.3	25.3	24.8	124.3	125.7	125.0	3.6	4.0	3.8	61.0	60.3	60.7
High	892.0	920.0	906.0	26.3	28.0	27.2	118.4	120.3	119.4	3.0	3.3	3.2	71.0	70.7	70.9
LSD	37.7	62.7	–	2.3	1.9	–	3.2	3.0	–	1.1	0.86	–	3.4	3.3	–
Date of sowing															
1 Jan	950.0	987.5	968.5	30.0	31.0	30.5	126.0	127.5	126.5	4.0	4.5	4.3	67.0	65.0	66.0
15 Jan	855.0	867.5	861.3	25.0	26.5	25.8	121.7	123.0	122.4	3.5	3.5	3.5	65.0	65.0	65.0
2 Feb	770.0	820.0	795.0	21.0	22.5	21.8	116.5	118.5	117.5	2.5	3.0	2.7	66.0	66.0	66.0
LSD	48.0	14.3	–	1.7	5.1	–	4.3	1.3	–	1.3	0.7	–	4.0	3.8	–
Rate × date	NS	NS	–	NS	NS	–	NS	NS	–	NS	NS	–	NS	NS	–
C.V	7.3	5.8	–	7.4	6.3	–	2.2	2.0	–	27.4	11.1	–	6.3	4.0	–

A= 1998/1999; B= 1999/2000; C= Average; NS= Not Significant

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Book review

M. F. SMALLWOOD, C. M. CALWERT, D. J. BOWLES (Eds): Plant Responses to Environmental Stress. 1999. BIOS Scientific Publishers. Guildford, UK. XXI+224 pages. ISBN 1-85996-192-4

Abiotic environmental stresses which limit plant distribution and productivity include low and high temperature, salinity and water deficit. Over the last century human activities have increased the level of environmental stress in the form of pollutants such as ozone and heavy metals, levels of UV light reaching the biosphere and salinity in irrigated areas. Plants are sessile and therefore have developed mechanisms to survive extreme environments in the vegetative stages of their life cycle. The molecular basis of these survival mechanisms is not only intrinsically interesting but may form the basis for enhanced plant productivity in currently marginal areas or to increase the cultivation latitude or season of agronomically important crops.

Plant Responses to Environmental Stress describes the latest research, critically reviewed by leading specialists in the field. Internationally recognized experts have contributed mini-reviews focusing on the effects of abiotic factors such as water, low and high temperature, salt and oxidative stress. The topics covered include the effects on protein function, the regulation of stress-responsive genes, and the molecular mechanisms involved in stress responses. The result is a fascinating and in-depth assessment of the current knowledge and of directions for future research, making this book an essential reference source for all those working on stress responses in plants.

J. SUTKA

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Kiss, G., Papp, I., Bakondi-Zámori, E., Gartner-Bánfalvi, Á. (1977): A szója fungicides magcsávázásának és rhizóbium oltásának együttes tanulmányozása. (Joint study of fungicide dressing and rhizobium inoculation in soybean.) *Növénytermelés,* **26,** 147–153.

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CANOPY TEMPERATURES AND EXCISED LEAF WATER LOSS OF TEF (*ERAGROSTIS TEF* [ZUCC.] TROTTER.) CULTIVARS UNDER WATER DEFICIT CONDITIONS AT ANTHESIS

A. TAKELE

MELKASSA RESEARCH CENTER, P.O. BOX 436, NAZRETH, ETHIOPIA

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This experiment was carried out to evaluate the canopy temperatures and excised leaf water loss (ELWL) of tef cultivars under water deficit conditions at anthesis and to demonstrate that these indices are reliable indicators of plant water stress. Twelve tef cultivars of similar maturity group but diverse origin were grown in each of two seasons under stressed and non-stressed conditions at anthesis. Mean cultivar canopy temperatures ranged from 33.2 to 34.9°C and 32.2 to 33.8°C in 1998 and 1999, respectively. There was also a significant difference in canopy temperature between treatments. The canopy temperature of stressed plants was 10.7% and 11.4% higher than that of non-stressed plants in 1998 and 1999, respectively. Under stress conditions the canopy temperature of the cultivars ranged from 33.6 to 36.7°C and from 33.1 to 37.6°C in 1998 and 1999, respectively, as compared to the non-stressed plants which ranged from 32.1 to 34.5°C in 1998 and from 29.7 to 31.9°C in 1999. There was a marked difference in mean excised leaf water loss (ELWL) values between the stressed and non-stressed treatments. There was also a differential response among tef cultivars for ELWL in response to the water deficit treatments during 1998 and 1999. Under non-stressed conditions the values of ELWL ranged between 1.5 g/g/h to 2.1 g/g/h in 1998 and 0.8 g/g/h to 1.7 g/g/h in 1999, whereas under stressed conditions the ELWL of the cultivars ranged from 1.0 g/g/h to 1.8 g/g/h and 0.7 g/g/h to 1.3 g/g/h in 1998 and 1999, respectively. The difference between the cultivars for both canopy temperatures and ELWL in response to the water deficit treatment was greater during 1999 than in 1998. It was concluded that both canopy temperatures and ELWL were suitable methods for the screening of drought resistant tef cultivars since differences between cultivars were detected.

Key words: canopy temperature, *Eragrostis tef*, excised leaf water loss

Introduction

Drought is a growing problem in many of the major crop-producing regions of Ethiopia. The problem is widespread in the semi-arid areas of the country. The main characteristics of the climate in these areas is that moisture is adequate for maximum crop growth very seldom and for very short periods (Ceccarelli, 1984). Tef is an important cereal food crop grown only in Ethiopia. It is widely grown in diverse agro-ecologies and currently tef production is increasing more than ever in semi-arid areas replacing sorghum and maize. This is because of its relative drought resistance and the fact, that it matures early when sorghum and maize usually fail. The main source of water supply for tef production in all agro-ecological zones in Ethiopia is rainfall. Although tef is

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usually planted when the soil is very wet, the stages of growth at which tef encounters frequent drought include the seedling, vegetative and reproductive stages. Farmers find that moderate drought stress at both the seedling and vegetative stages is advantageous, as the yield of tef is not reduced and may even be increased as a result of compensatory growth and the development of an increased number of tillers when rainfall recommences. However, drought occurring during the anthesis and grain filling stages is considered to be critical since this drought substantially reduces yield. This is because drought reduces the translocation of non-structural carbohydrates from the source to the sink and consequently reduces grain yield.

As drought resistance is one of the many traits needed in a crop cultivar, progress in breeding for drought resistance in crop plants seems to be a practical and economical solution. However, the genetic improvement of drought resistance in crop plants requires the identification of relevant morphological and physiological drought resistance traits as selection criteria (Blum et al., 1982). Although the national tef improvement programme has attempted to develop drought-resistant tef cultivars, progress has been very slow. This is because knowledge based on the morphological and physiological traits for drought resistance in tef is lacking. The limited research efforts carried out in the past indicated that there is tremendous morphological and physiological variability in tef genotypes (Takele, 1997; Shiferaw and Baker, 1996a; Ayele, 1993). The work of Shiferaw and Baker (1996b) demonstrated that no single physiological trait had sufficient reliability in determining the response of tef cultivars to drought stress. Ayele (1993) identified excised leaf water loss (ELWL) as a simple and reliable selection criterion in tef breeding for drought resistance. Other studies (Clarke and McCaige, 1982; Kirkham et al., 1980; Sandhu and Laude, 1958) have indicated the reliability and efficiency of excised leaf water retention as a selection methodology for drought resistance. Previous research results also revealed that measurements on the canopy temperature of crop species exposed to drought was a fast and reliable tool for the selection of crop varieties for drought resistance (Blum et al., 1982; Singh and Kanemasu, 1983).

This study was therefore carried out to evaluate canopy temperature and excised leaf water retention as selection methodologies for drought resistance in tef.

Materials and methods

The study was conducted at Melkassa Agricultural Research Center farm, located at Nazreth, Ethiopia (longitude 39°21' E and latitude 8°24' N) on a sandy loam soil during the dry season of 1998 and 1999. The area lies at an altitude of 1550 m.a.s.l. The mean maximum and minimum temperatures during the experiment were 28.9°C and 13.9°C, respectively. The crops were planted on 25 and 28 March 1998 and 1999, respectively. The treatments consisted of twelve tef cultivars selected on the basis of their yield performance in the national yield trials under rainfed conditions. These were 1) Dz-01-354, 2) Dz-cr-37, 3) Dz-01-2054, 4) Dz-01-1231A,

5) Dz-01-2053, 6) Dz-01-196, 7) Dz-01-1278, 8) Dz-01-1444, 9) Dz-01-234, 10) Dz-01-1717, 11) Dz-01-130 and 12) Dz-01-2089. This study concentrated on drought occurring at anthesis in an attempt to simulate the drought commonly suffered by the crop in the dryland areas of the country. Thus, the plants were subjected to drought stress at anthesis, together with an irrigated (control) treatment. Each cultivar was broadcasted on a 4 m^2 plot in a split plot design, with drought levels as the main plot and cultivars as sub-plots in three replications.

Canopy temperatures were measured between 1200 and 1400 h with a hand-held infrared thermometer (AG-42 Telatemp Corporation, California, U.S.A.). Measurements of canopy temperature were made when the tef canopies closed and only under unobstructed direct solar beam conditions. The canopies were viewed at an angle of 45° to avoid interference from the soil surface.

Excised leaf water loss (ELWL) measurements were made by sampling five young fully expanded leaves from each plot, which were put in plastic bags in an ice-box to control transpirational water loss during transfer to the laboratory. After recording the fresh weight the samples were placed for 2 h in a controlled temperature chamber at 30°C, weighed and then oven dried at 80°C for 24 h. After a further weighing, the ELWL was computed using the formula:

$$\frac{\text{FLW} - \text{2DLW}}{\text{24DLW}}$$

where FLW is fresh leaf weight, 2DLW is dry leaf weight after 2 h and 24DLW is dry leaf weight after 24 h.

At maturity the grain yield was determined by harvesting the whole of each plot. The data were then subjected to statistical analysis using the MSTATC program. The F-ratio was used to test overall differences between the various treatment means in the analysis of variance table. To perform a pair-wise comparison of all treatment means, one of the multiple range tests, Least Significant Difference (LSD) was used. Simple correlations were calculated between grain yield and canopy temperature and ELWL across genotypes in the water deficit treatment using the 1999 data.

Results and discussion

The data of mean canopy temperature during 1998 and 1999 showed that there were significant ($p<0.05$) differences between the tef cultivars. The mean cultivar canopy temperatures ranged from 33.2 to 34.9°C and 32.2 to 33.8°C in 1998 and 1999, respectively. There was also a significant difference in mean canopy temperature between the water deficit treatments. The canopy temperature of the stressed plants was 10.7% and 11.4% higher than that of non-stressed plants in 1998 and 1999, respectively. The interaction means of the water deficit treatment and cultivar on canopy temperature during 1998 and 1999 are presented in Figs. 1a and 1b, respectively. Water deficit treatment had no significant effect on the canopy temperature of any of the cultivars in 1998, but in 1999 water deficit treatment caused a significant increase in canopy temperature in all cultivars. Under stress conditions the mean canopy temperature ranged from 33.6 to 36.7°C and from 33.1 to 37.6°C in 1998 and 1999, respectively, as compared to the non-stressed treatment, where it ranged from 32.1 to 34.5°C in 1998 and from 29.7 to 31.9 °C in 1999. The absence of a significant interaction between the water deficit treatment and the cultivars in 1998 was due to the interference of rainfall two to three days before the data were recorded. Although there was no water deficit effect on the cultivars the

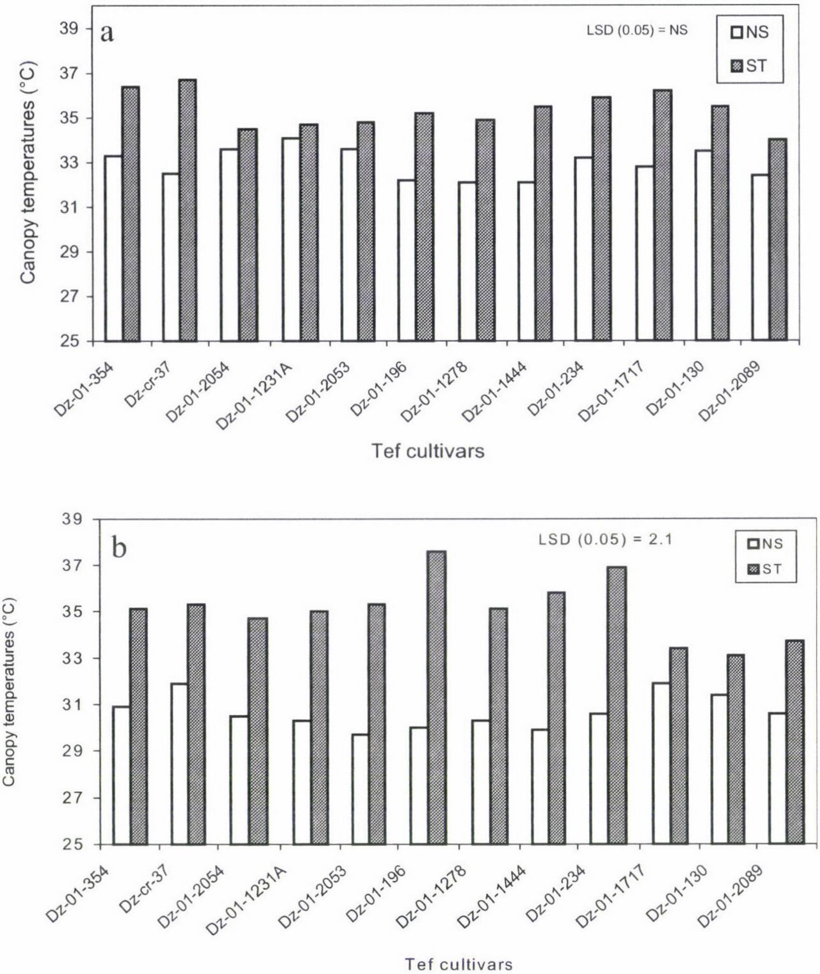

Fig. 1. Canopy temperatures (°C) of twelve tef cultivars under water deficit conditions at anthesis during a: 1998 and b: 1999

data demonstrated that canopy temperature was increased by water deficit within a range of 2% to 13% among the cultivars as compared with the control. The increase in canopy temperature due to water deficit treatment during 1999 was greater than during 1998, the increase being within a range of 5% to 25% among the cultivars as compared with the control. During 1998 under water deficit conditions, Dz-cr-37, Dz-01-1444 and Dz-01-1717 had the highest percentage increase in canopy temperature, with increases of 13%, 10.6% and 10.4% compared to the control, respectively, and Dz-01-1231A had the lowest, 2% increase in canopy temperature relative to the control. During 1999 the highest percentage increase in canopy temperature was recorded for Dz-01-196 (25%), Dz-01-234 (20.6%) and Dz-01-1444 (19.7%) and the lowest for Dz-01-130 (5%). The increase in the canopy temperature of stressed cultivars indicated that there was a reduction in the transpirational water loss probably due to stomatal closure. Hatfield et al. (1987) indicated that the canopy temperature of each cultivar depended on the amount of water remaining in the soil. Thus, under stress conditions there was no water available for transpirational cooling, thereby increasing the canopy temperature of the stressed plants. Under the conditions of this experiment, therefore, cultivars with higher temperatures are advantageous for conserving water for sensitive development stages such as anthesis during periods of water deficit. The results of Blum et al. (1982) showed that canopy temperature is useful as a screening technique for dehydration avoidance.

ELWL was significantly ($P<0.05$) influenced by the water deficit treatments during 1998 and 1999. The data indicated that the non-stressed plants had poor water retention capacity in contrast to the stressed plants which had superior water retention capacity, suggesting that under water deficit conditions there was a reduction in transpirational water loss due to stomatal closure. The mean ELWL values were 1.8 g/g/h and 1.4 g/g/h in 1998 and 1.4 g/g/h and 1.0 g/g/h in 1999 for non-stressed and stressed treatments, respectively. The interaction means of the water deficit treatment and cultivars on ELWL during 1998 and 1999 are presented in Figs. 2a and 2b, respectively. Evidently, water deficit caused a marked reduction in ELWL in all the cultivars during both 1998 and 1999. When the reduction in ELWL as a result of water deficit treatment was considered as a percentage of the control, it was found to range from 0 to 47% over the cultivars during 1998 and from 15% to 47% during 1999 as compared to the control. During 1998, under non-stressed conditions the values of ELWL ranged from 1.5 g/g/h to 2.1 g/g/h compared to the stressed plants, which had values between 1.0 g/g/h and 1.8 g/g/h, while during 1999, the values of ELWL ranged from 0.8 g/g/h to 1.7 g/g/h and from 0.7 g/g/h to 1.3 g/g/h under non-stressed and stressed conditions, respectively. The results of this study are in contrast to those of Ayele (1993) who reported a non-differential response among cultivars to water deficit treatment at anthesis. This may be due to the following two reasons. There was a difference in the intensity of water deficit. The tef cultivars in this study had a restricted water supply and were subjected to severe water deficit at anthesis, a phenomenon which often occurs in the major tef-growing dryland areas of Ethiopia. Secondly, the soil type where this study was performed was sandy loam, which has poor water-holding capacity. When soils of this type are subjected to water deficit, the soil surface quickly dries out as a result of evaporation and tef cultivars with shallow root length will subsequently show signs of stress. The differences in the water retention capacity of the tef cultivars in this study may also be due to differences in their stomatal density. The responses of the cultivars to water deficit treatment were inconsistent over the two years. For example, under water deficit conditions, cultivars Dz-01-1717, Dz-01-1231A, Dz-01-234 and Dz-01-2089 showed poor water retention capacity in 1998 and Dz-01-1444 in 1999, whereas Dz-cr-37 and Dz-01-1278 showed superior water retention capacity in 1998 and

Dz-01-234, Dz-cr-37, Dz-01-196, DZ-01-1278 and Dz-01-2089 in 1999. This may be due to the diversity of the environmental conditions during the years of study. Under such conditions, therefore, a combination of tests may be needed to rank resistant and susceptible groups of cultivars (Shiferaw and Baker, 1996). However, Dz-cr-37 and Dz-01-1278 consistently had superior water retention capacity in both years. The work of Clarke and McCaige (1982), Kirkham et al. (1980) and Sandhu and Laude (1958) indicated superior water retention capacity to be associated with drought-resistant cultivars rather than with drought-susceptible cultivars.

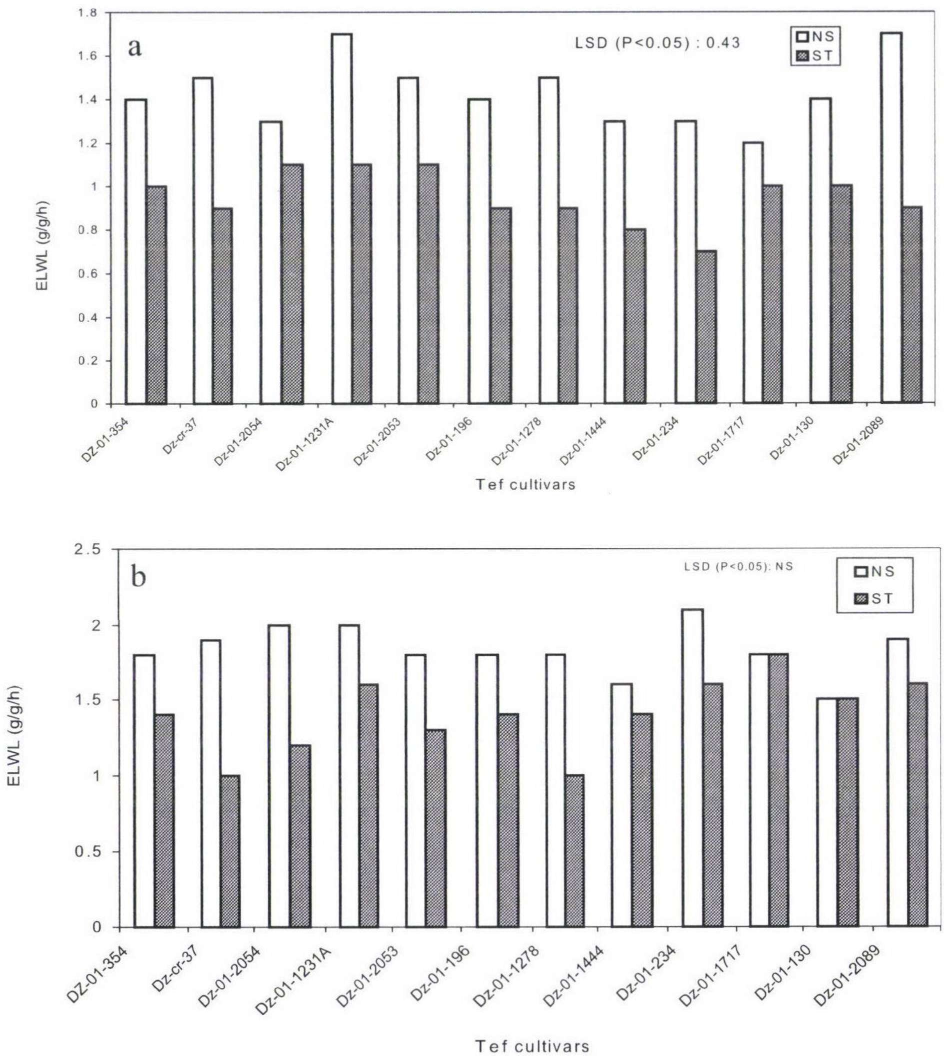

Fig. 2. ELWL (g/g/h) of twelve tef genotypes under water deficit conditions at anthesis during a: 1998 and b: 1999

Grain yield was significantly ($P < 0.01$) different between cultivars during 1998 and 1999. The mean grain yield of the cultivars ranged from 0.44 t/ha to 0.74 t/ha and from 0.37 t/ha to 1.01 t/ha during 1998 and 1999, respectively. The highest mean grain yields of 0.74 t/ha and 1.01 t/ha were produced by Dz-01-2054 during both 1998 and 1999. The water deficit treatment significantly ($P < 0.01$) reduced the mean grain yield by 77% and 69% during 1998 and 1999, respectively. The interaction means of the cultivars and water deficit treatments on grain yield are presented in Figs. 3a and 3b. Analysis of variance indicated that the interaction effect of cultivars by water deficit treatment was significant ($P < 0.001$) in both years, indicating genotypic variability among tef cultivars in response to the water deficit treatment. Thus, under water deficit conditions the reduction in grain yield of the cultivars ranged from 57% to 87% and from 52% to 86.2% during 1998 and 1999, respectively. Among the cultivars the highest reduction in grain yield occurred in Dz-01-234, Dz-01-1717 and Dz-01-130 in 1998 and in Dz-01-234, Dz-01-1278 and Dz-01-196 in 1999. In non-stressed environments tef showed ample genetic variation in both grain and biomass yield (Ketema, 1997), whereas under water deficit conditions similar results were reported under glasshouse (Takele, 1997) and field conditions (Ayele, 1994–95). The existence of genetic variability in grain yield and other agronomic characteristics indicates the possibility of improving the yield potential of tef cultivars under water deficit conditions.

The correlation coefficients between grain yield and canopy temperature was significant and negative ($r = -0.78$) indicating that under water deficit conditions warmer cultivars are more drought resistant, while cooler cultivars have greater water use efficiency. Similarly, the grain yields of the cultivars under water deficit conditions were significantly and negatively ($r = -0.59$) correlated with mean ELWL, again suggesting that under water deficit conditions cultivars with poor water retention capacity are high yielding, while those with superior water retention capacity are more drought resistant. The canopy temperatures of the cultivars were significantly and negatively ($r = -0.53$) correlated with mean ELWL. These results again confirmed that warmer cultivars are superior in water retention capacity and thus more drought resistant.

Conclusions

The data of Shiferaw and Baker (1996b) demonstrated that the mode of adaptation to drought stress in tef is avoidance mechanism. The crop may avoid drought stress by conserving water either by reducing the area of transpiration through a reduction in leaf size or by stomatal closure. These have been shown by measurements of leaf area and stomatal conductance. From the results of this study it was observed that canopy temperature and excised leaf water loss are potential selection criteria for screening for drought avoidance in tef cultivars during periods of early rainfall cessation and under conditions of severe drought

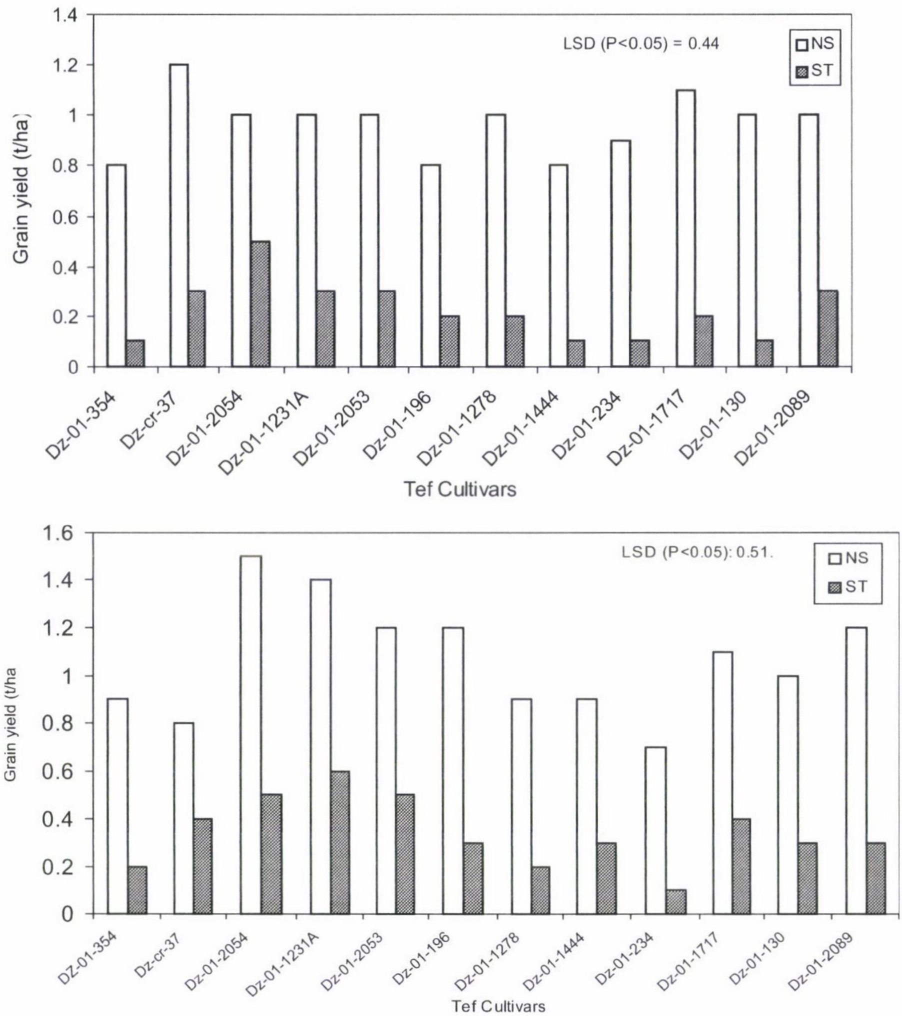

Fig. 3. Grain yield (t/ha) of twelve tef genotypes under water deficit conditions during a: 1998 and b: 1999

at anthesis. Further, the response of the canopy temperatures and water retention capacity of tef cultivars under water deficit treatment suggests maximum stomatal resistance, which would lead to reduced photosynthesis and thus growth. This further indicated that tef exhibited extreme drought avoidance mechanisms. Therefore, cultivars with intermediate levels and a pattern of stomatal response that would enhance greater water use efficiency need to be sought. The advantage of measuring canopy temperatures and excised leaf water loss over the others is that both techniques are fast, permitting the screening of large numbers of samples and allowing repeated data recording in a relatively short period of time.

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GROWTH AND ENERGY CONTENT OF THREE FORAGE GRASSES FROM THE MIDDLE EAST RANGELANDS

A. K. HEGAZY[1] and A. A. EL-KHATIB[2]

[1]DEPARTMENT OF BOTANY, FACULTY OF SCIENCE, CAIRO UNIVERSITY, GIZA, EGYPT
[2]DEPARTMENT OF BOTANY, FACULTY OF SCIENCE, SOUTH VALLEY UNIVERSITY, SOHAG, EGYPT

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This study was conducted on three naturally growing populations of the perennial grasses *Panicum turgidum* Forssk, *Lasiurus scindicus* Henrard and *Pennisetum divisum* Henrard in Egypt, south west Saudi Arabia and Qatar. Vegetative reproduction occurs by rhizome growth and sexual reproduction by tillering, where each tiller may end with a spike. The failure of sexual reproduction is mainly related to the extremely arid conditions and the overgrazing of flowering branches or seeds before dispersal. Phenological behaviour varied among species and differed within the same species in the three study areas. The earliness/lateness and narrow/wide spectrum of the active phenological phases (vegetative growth, flowering and fruiting) were species-dependent rather than locality-dependent. The phenophases of the three species in Saudi Arabia and Qatar proceeded at a faster rate than in Egypt. The plants collected from Saudi Arabia and Egypt attained higher energy content than those collected from Qatar. The overall energy content of *P. turgidum* (over 6 kcal. g^{-1} dry weight) is higher than that of *L. scindicus* and *P. divisum* (less than 6 kcal. g^{-1} dry weight). During the peak flowering/fruiting time, the fruits and rhizomes of the three species showed higher energy content than the roots and shoots. When the growth characteristics and energy content were taken as a measure of the grazing value, *P. turgidum* had better value as a forage plant than the other two species.

Key words: phenology, brachyblasts, dolychoblasts, leaf area, water content, caloric value

Introduction

Despite the recently expanding agricultural, industrial and social developments in the Middle East countries, rangeland ecosystems are still the main source of livelihood in some localities for up to 30% of the population. Rangelands are also unrivalled ecosystems in the Middle East as a source of forage for free-ranging wild and domestic animals, as a source of plant material for different purposes (e.g. wood, medicine, thatching, matting, etc.), for wildlife diversity, and for recreational opportunities as open spaces with scenic beauty.

The deterioration of rangelands in the Middle East has been generally attributed to a number of factors: (a) Scarce and sporadic rainfall; (b) Frequent drought; (c) Overgrazing; (d) Overcutting of range shrubs and bushes for fuel; (e) Unmanaged carrying capacity and absence of grazing rotations; and (f) Expansion of desert reclamation for agriculture at the expense of rangelands. Previous studies on rangelands in the Middle East are few, fragmented and deal with traditional subjects such as flora, vegetation and community ecology of some forage plants, rather than dealing with rangelands as integrated ecosystems that need sustainable use and proper management (Migahid and El-Shourbagui, 1958a, b; Draz, 1969, 1979; Abdel Rahman, 1986; Orshan, 1986; Zahran and Younes, 1990).

Since the diversity of plants is the major foundation for rangelands, the development of management strategies and plans for the sustainable use of rangeland plants requires information about the ecology of the grazing species in different localities. This involves the assessment of the grazing value of the species at different localities, where environmental variability and ecosystem structure and function are very dynamic. In the Middle East region, forage plant growth and its grazing value are functions of the species ecology, the frequent drought and the grazing animals, and the sustainable use of rangelands is an important chanllenge (Hegazy, 2000, 2001).

Information about the basic ecology and grazing value of most forage plants in the region is limited. Only a few fragmented studies on some common species are found in the region (Suliman, 1988; Le Houerou, 1997; Omar et al., 1999; Thomas, 1999). This study focuses on the investigation of the phenology, growth and energy content of three common rangeland grass species in deserts of the Middle East. An attempt will be made to rank the three grasses in order of importance to livestock based on the assumptions that: (1) the length of the active phenophases, (2) the number of shoots produced, (3) the average leaf area, (4) the plant water content, and (5) the energy content of the aboveground plant parts can individually and collectively be used as a measure of grazing value.

Materials and methods

Study sites

The study was carried out: (1) in the eastern section of *wadi* Hagoul, eastern desert, Egypt during January to December 1997; (2) along Suada Nathil road, south west Qatar during January to December 1994; and (3) around Baysh town along the El Darb-Gizan road, south west Saudi Arabia during September 1991 to July 1992. Site locations are shown in Figure 1.

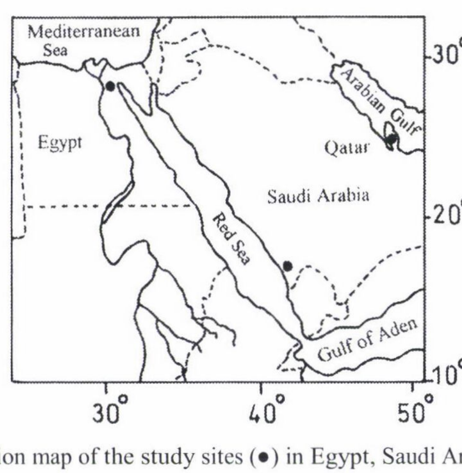

Fig. 1. Location map of the study sites (•) in Egypt, Saudi Arabia and Qatar

Environment

The region lies within a homo-climatic and homo-ecological, extremely arid belt with less than 80 mm rainfall per year (Evenari, 1981; Le Houerou, 1986; Orshan, 1986; Hegazy, 1999). The characteristic features of the climate are the high temperature and low relative humidity of the air. Rainfall shows extreme quantitative, temporal and spatial irregularity, being uncertain, variable and unpredictable. The region has a pronounced winter rainfall season with a few rainy days. Another typical feature is the irregular occurrence of sudden showers (extreme rain pulses) which produce large quantities of water falling in a short time at high intensities. The soils are sandy, alkaline and rich in carbonate content, but very poor in organic matter, with low electric conductivity (Hegazy, 1995, 1996; El-Demerdash et al., 1995). The monthly rainfall during the years of observation at the three study sites is shown in Table 1.

Table 1

Monthly rainfall (mm) during the years of observation at the three study sites in Qatar (Doha Station), Saudi Arabia (Gizan Station) and Egypt (Kattamia Station)

Study site	Jan.	Feb.	March	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Qatar	1.8	14.0	22.0	0.6	–	–	–	–	–	–	1.5	7.9	49.6
Saudi Arabia	13.0	15.2	23.8	–	2.5	–	–	–	–	0.3	–	10.6	54.4
Egypt	10.7	18.5	16.1	–	–	–	–	–	–	1.4	–	0.8	47.6

Study species

The three study species *Panicum turgidum* Forssk, *Lasiurus scindicus* Henrard and *Pennisetum divisum* Henrard are long-lived perennial desert grasses. The three species are mainly grazed by domestic camels, sheep and goats. They have woody culms arising from stout rhizomes or rootstocks and grow up to a height of 1.5 metres or more. The culms may be simple or branched. The root systems are widely spreading and deeply penetrating. They were found to grow in large thickets on both deep sandy and gravel soils in the main drainage channels and valleys in which there is active deposition and building up of transported soils. The three species show high resistance to drought and grazing pressure. They are tussock-forming plants as they leave some undecomposed litter which helps in building the soil. The plants act as obstacles to the drifted or wind-blown sands and as a result, the heaped up sand forms phytogenic hillocks (Evenari, 1981; Le Houerou, 1986) around their base. The three species may grow in association with each other and the grazing animals have different preferences. The growth of the studied species is characterized by the production of two shoot types: (1) *Brachyblasts*, which are dwarf shoots (sprouts) developing at various heights on the old culms, and are characterized by a slow growth rate. They may or may not develop inflorescences during the first season of their emergence. They continue to develop in all seasons, but with different intensities. They are usually reproductive branches that function for more than one season. They may produce inflorescences or branched culms; (2) *Dolychoblasts*, which are long vegetative shoots, usually develop during the growing season either from the culms (elongation of the brachyblasts) or from rhizomes. They are characterized by a fast growth rate. They do not usually produce inflorescences during the first season of their development. Their growth gives rise to new culms. All the old branches are dolychoblasts.

Phenology

Periodic observations on phenological phases were carried out on 10 tagged adult plants. Observations were taken according to the technique used by Hegazy and Eesa (1991) which provides numerical ratings of closely defined phenological phases. The phenological index denotes the base score of the phenophase and the percentage to the nearest 10% of the subsequent phenophase. Five phenophases were recorded, with base scores of 1.0 = vegetative growth phase;

2.0 = flower bud and flowering phase; 3.0 = fruiting and seed dispersal phase; 4.0 = senescing and dormancy phase; and 5.0 = incidental or out-of-season sprouting phase. The phenophases were defined subjectively when making the observations.

Growth

Plant growth was measured by counting the number of sprouting shoot branches (brachyblasts and dolychoblasts) and measuring the leaf area on 10 tagged individual plants covering different size classes. The observations were carried out on a two-monthly basis. Sprouting was calculated as the number per 100 dm^3 of plant crown volume. The crown volume, V, was calculated by the formula:

$$V = 4/3\ \pi\ a\ b^2$$

where a = half crown diameter and b = shoot length (Ludwig et al. 1975).

The leaf area A was measured on a two-monthly basis by the formula:

$$A = 0.905\ LB$$

where L = leaf length and B = leaf breadth, with B taken as L/2 (Sestak et al. 1971). The leaf area was transcribed as cm^2 per 100 g shoot fresh weight.

Water content

The water content of the emerging shoot branches was determined from fresh and oven dry weights. The material was oven dried for 48 h at 80°C. The water content, C, was calculated as a percentage of fresh weight by the formula;

$$C = [(W_1 - W_2) / W_1]\ 100$$

where W_1 = fresh weight and W_2 = oven dry weight.

Energy content

The plants were carefully excavated at the fruiting phase and subsequently partitioned into roots, rhizomes, culms, live leaves and fruits. The energy content of the plant organs was determined by wet oxidation in potassium dichromate in sulphuric acid. The materials were thoroughly dried at 60°C, pulverized and stored for a few days in a desiccator until analysing following a method adopted by Wroblewski (1977).

Grazing value

The grazing value of the species was considered subjectively by comparing the individual aspects of different plant measurements including: length of active vegetative and flowering phenophases, number of the grazable shoots (brachyblasts and dolychoblasts), leaf area, water content, and energy content of grazable plant parts. Species with comparatively high values were considered as having high grazing value. The sequence of species from the highest to the lowest value was taken as a measure for ranking the value of the species as a forage plant.

Statistical analysis

Differences were tested statistically by ANOVA following Snedecor and Cochran (1967).

Results

Phenology

Although the initiation of active vegetative growth varied among species and sites, dormancy was broken during late November/early December. In Egypt and Qatar, *Panicum* plants started their vegetative growth in November, earlier than the plants in Saudi Arabia. In contrast, *Lasiurus* started vegetative growth in Saudi Arabia earlier than in Egypt and Qatar (Fig. 2). The vegetative growth of *Pennisetum* started during January in Saudi Arabia and Qatar, and during early spring in Egypt. In the three species, incidental vegetative sprouts were developed during autumn in the different localities. Vegetative sprouting started in *Panicum* and *Lasiurus* earlier than in *Pennisetum,* where sprouting continued to the mid-winter season. The initiation of flower buds and the flowering of *Panicum* and *Lasiurus* started during February in Saudi Arabia and during March in Qatar and Egypt. For *Pennisetum*, flowering started during March in Saudi Arabia, April in Qatar and May in Egypt. In all species, fruiting started about two or three weeks later than the flowering initiation. The fruiting period reached the optimum during May and June. The dormancy of all the species started earlier in Saudi Arabia and Qatar than in Egypt. In Egypt, *Lasiurus* attained a lengthy dormancy period of up to 6 months from late July to early January.

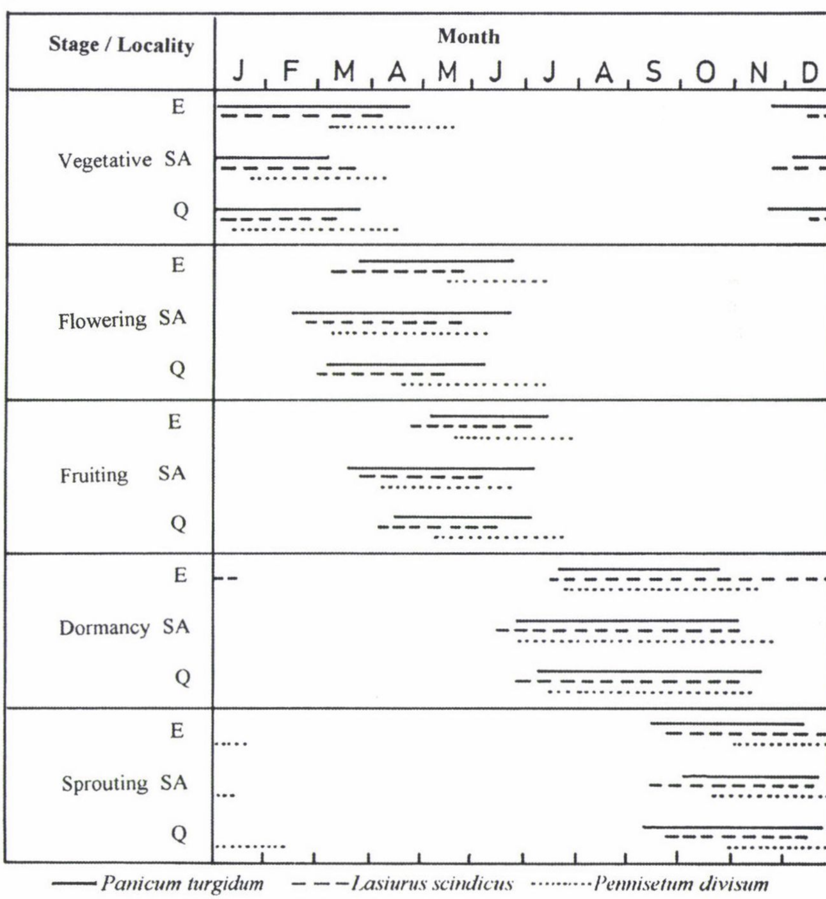

Fig. 2. Phenological spectrum of the three species investigated at different sites. E = Egypt, SA = Saudi Arabia and Q = Qatar

The average phenological indices demonstrate the similarities and variations in the length of the phenophases maintained by the three species at different sites (Fig. 3). Although the species varied considerably with regard to the length of time involved in a given phenophase, they all showed general consistency in the sequence of phenophases. The length of the vegetative period ranged from three months in *Pennisetum* to five months in *Panicum*. Flowering and fruiting occurred over a time period of four months in all species. The dormancy period ranged from four to six months. In general, the time of initiation for particular phenophases varied considerably among the species at different sites.

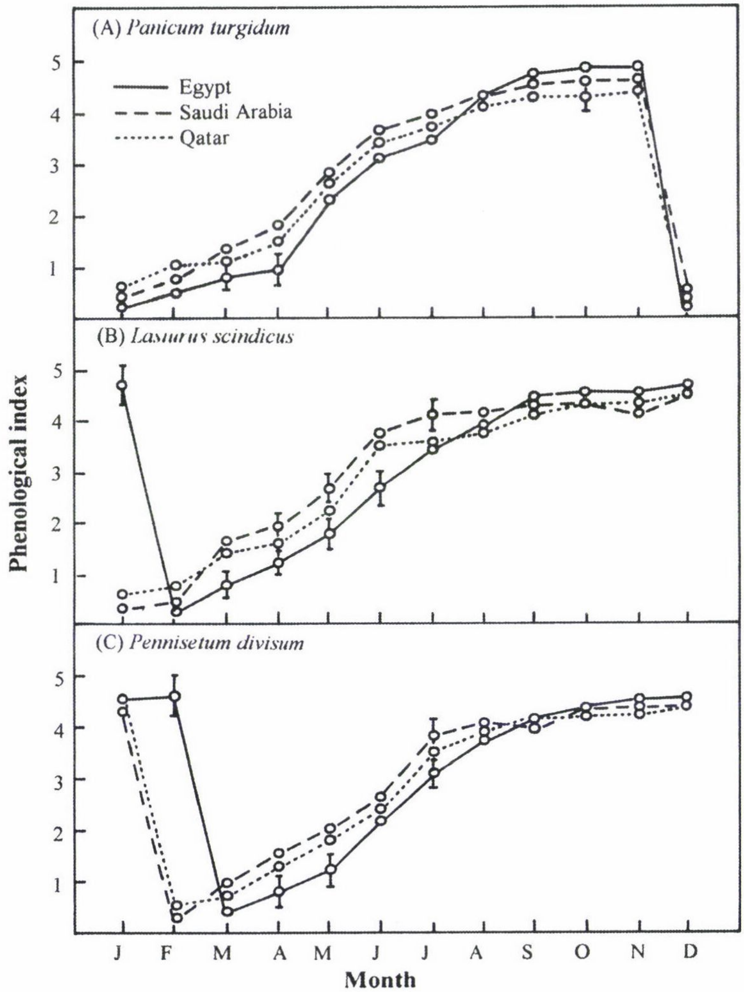

Fig. 3. Plant phenological index of the three species investigated at different sites. Mean values with standard deviations (vertical bars) are significantly different from the other sites at the 0.05 level (n = 10)

If the length of the active phenophases (vegetative, flowering and fruiting phases) is taken as a measure of plant grazing value, the following sequence (from highest values to the lowest) of the species was observed: *Panicum turgidum* > *Lasiurus scindicus* > *Pennisetum divisum*.

Growth

Most plants of the three species at the different sites produced brachyblasts (dwarf branches) continuously throughout the year. The number of brachyblasts reached peak values during March to May (Fig. 4 A1, B1 & C1). Thereafter, brachyblast emergence decreased throughout the summer, with minimum values in September. The total number of brachyblasts produced per 100 dm^3 of crown volume per year in *Panicum* reached 103 in Egypt, 122 in Saudi Arabia and 72 in Qatar. Both *Lasiurus* and *Pennisetum* produced less than half as many brachyblasts as *Panicum* at the different sites. The brachyblast production by all the species was better in Egypt and Saudi Arabia than in Qatar.

The number of dolychoblasts (long branches) produced by all the species was much lower than that of brachyblasts at the different sites (Fig. 4 A2, B2 & C2). The average number of dolychoblasts produced per 100 dm^3 of shoot size per year in *Panicum* was 22 in Egypt, 20 in Saudi Arabia and 14 in Qatar. In *Lasiurus*, the average numbers of dolychoblasts produced per 100 dm^3 of shoot size per year were 13, 8 and 7 in Egypt, Saudi Arabia and Qatar, respectively. *Pennisetum* produced intermediate amounts of dolychoblasts between the other two species. At all sites, neither *Lasiurus* nor *Pennisetum* produced dolychoblasts during mid-summer. For *Panicum*, dolychoblasts continued to emerge all the year round in Egypt, while production stopped during mid-summer in Saudi Arabia and Qatar.

If the number of brachyblasts and dolychoblasts produced is taken as a measure of plant grazing value, the following sequence (from highest values to the lowest) of the species was observed: *Panicum turgidum* > *Pennisetum divisum* = *Lasiurus scindicus*.

The sequence of leaf production seems to be more synchronous for all species investigated at the different sites. The average leaf area increased during winter and attained the highest values in early spring and the lowest values in early autumn (Fig.5 A1, B1 & C1). In Egypt and Saudi Arabia, the three species produced higher leaf area than in Qatar. The leaf area production in *Panicum* was more than twice that of *Lasiurus*, while *Pennisetum* attained an intermediate value. *Lasiurus* did not produce any new leaves during September in Saudi Arabia, while the same was true for *Pennisetum* in Qatar. The average maximum leaf area produced by *Panicum* was 158 cm^2 per 100 g shoot fresh weight in Saudi Arabia, 141 cm^2 in Egypt and 82 cm^2 in Qatar.

The water content of the shoots in the three species fluctuated within a narrow minimum-maximum difference range. The plants attained the highest shoot water content during winter and early spring, while the lowest values were recorded during early autumn (Fig. 5 A2, B2 & C2). *Panicum* exhibited higher shoot water content than *Lasiurus* and *Pennisetum* at all study sites. The average maximum values of shoot water content for all species at different sites ranged between 48% and 58%, while the minimum values ranged between 38% and 46% of the fresh weight. The average minimum-maximum difference values of shoot water content for the three species ranged between 8 and 15 at the different study sites.

If the average leaf area and water content are taken as a measure of plant grazing value, the following sequence (from highest values to the lowest) of the species was observed: *Panicum turgidum* > *Pennisetum divisum* > *Lasiurus scindicus*.

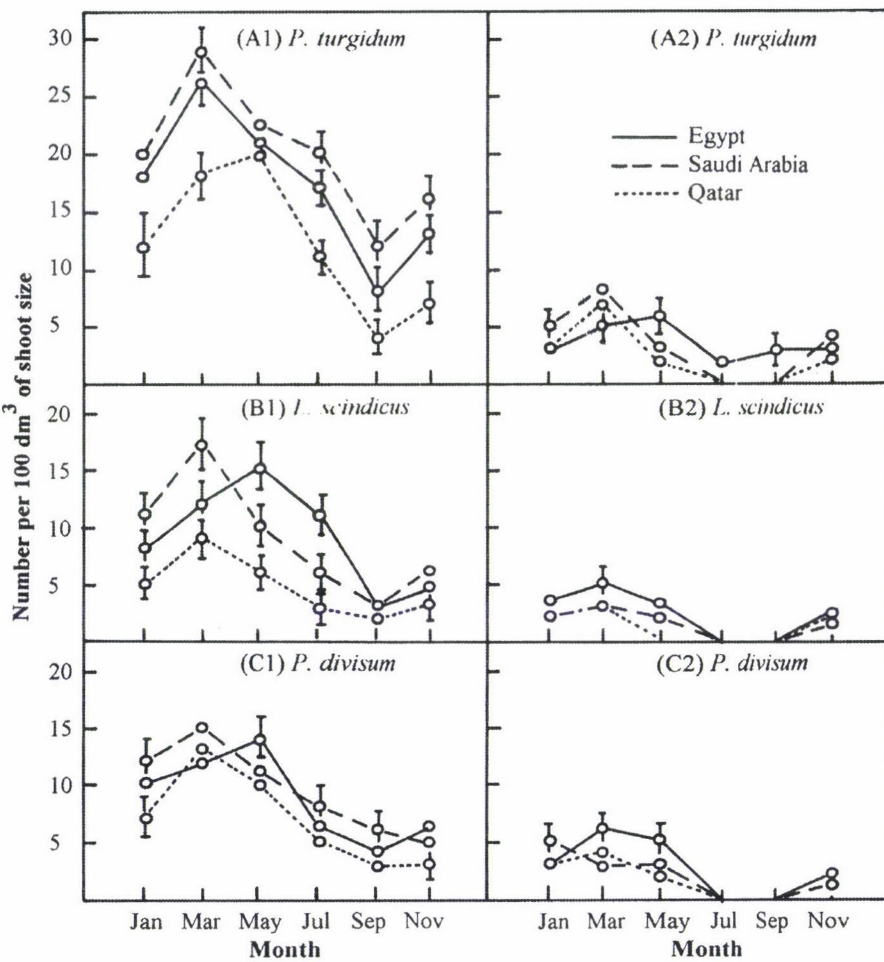

Fig. 4. Number of emerging brachyblasts (A1, B1 & C1) and dolychoblasts (A2, B2 & C2) in the three species investigated at different sites. Mean values with standard deviations (vertical bars) are significantly different from the other sites at the 0.05 level (n = 10)

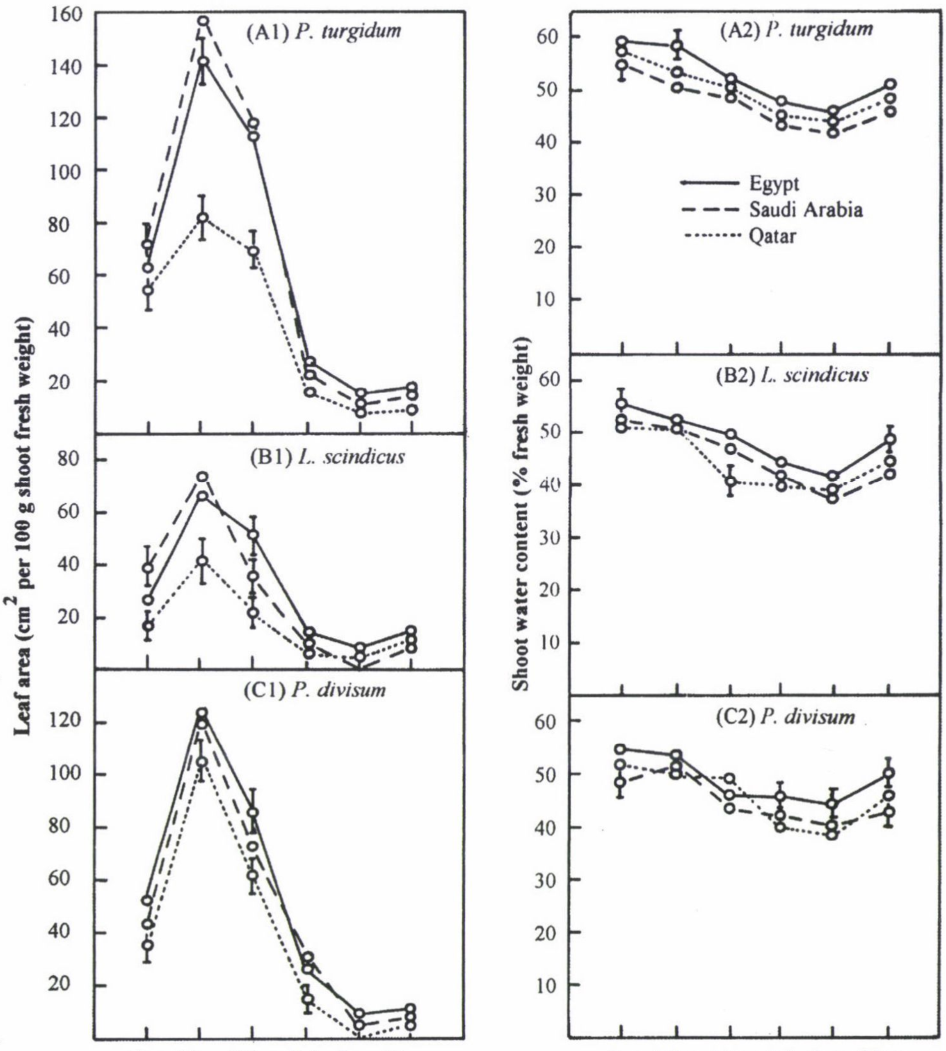

Fig 5. Variation of leaf area (A1, B1 & C1) and shoot water content (A2, B2 & C2) in the three species investigated at different sites. Mean values with standard deviations (vertical bars) are significantly different from the other sites at the 0.05 level (n = 10)

Energy content

A comparison of the energy content in the three species at different sites showed inter-specific and intra-specific variations. In comparison with the other species, *Panicum* showed the highest average energy content, which varied among the plant organs and depended on the locality (Fig. 6). The energy values of the roots were the lowest among plant organs and were non-significantly different between species at different sites. At the species level, fruits had higher energy content than the other plant parts. The highest energy content of fruits amounted to 7.2 kcal g^{-1} dry mass in *Panicum*, 4.8 kcal in *Pennisetum* and 3.9 kcal in *Lasiurus*. On the other hand, the rhizomes showed higher energy content than the culms and leaves in all studied species.

If the average energy content of grazable plant parts (fruits, leaves and culms) is taken as a measure of plant grazing value, the following sequence (from highest values to the lowest) of the species was observed: *Panicum turgidum* > *Pennisetum divisum* = *Lasiurus scindicus*.

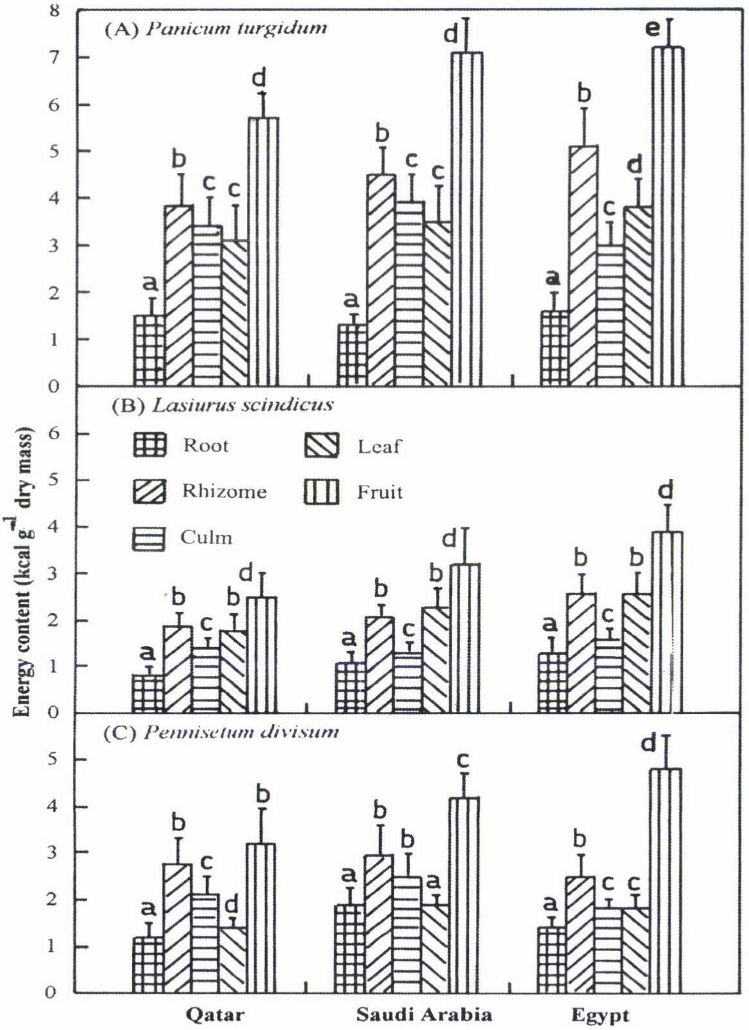

Fig. 6. Variation of energy content (kcal.g^{-1}dry mass) in different plant organs of the three species investigated at different sites. Values are means ± S.D., n = 5. Bars labelled with similar letters at the same site are significantly different at the 0.05 level

Discussion

Differences in localities did not alter the general growth and energy content patterns of the three species studied. The timing of phenological events differed more between species than within species at different localities. This flexibility may account for the species having a relatively wide range of distribution (Lee, 1971; Lieth, 1974; Hoffman and Walker, 1980; Estabrook et al., 1982; Rathcke and Lacy, 1985; Hegazy et al., 1998; Hendricksen et al., 1999; Omar et al., 1999). The continued vegetative growth and incidental sprouting which extends almost throughout the year is an advantageous strategy for plant survival, for coping with the environmental limitations and for compensating for heavy grazing stress. This behaviour enables the studied plants to utilize favourable periods during the dry summer and autumn.

The continuous grazing of emerging shoots (brachyblasts and dolychoblasts) may stimulate the initiation of secondary buds. This results in the continuous sprouting of new branches. This process is essential for the survival and longevity of plants under drought stress and heavy grazing. Since active and less active periods alternate around the year, the production of dolychoblasts occurs during the active growth periods, while the brachyblasts sprout throughout the year.

The analysis of the growth of the investigated species indicated the occurrence of two grazing periods; *first*, the winter/spring period (from January to May) and *secondly*, the summer/autumn period (from June to December). In the first grazing period, the plants produce large amounts of grazable material, i.e. vegetative and reproductive shoots having a relatively high water content. In the second grazing period, both the amount of grazable material and its water content are much lower than in the first period. Accordingly, the carrying capacity and the amount of supplementary feed must be estimated according to the amount of grazable material rather than the vegetation type and its component flora.

Measurements on the water content of the grazable shoots revealed that the living plant material of the species studied contained from 50% to 58% average water content during winter and spring, and from 38% to 50% during summer and autumn. Differences in water content between the maximum and minimum values were in the order of 8% to 15%, which is considered relatively low (Larcher, 1980). This indicates the occurrence of two periods of relatively stable water content, parallel to the two grazing periods, an important asset for the grazing value of the species in the rangelands of arid regions.

The high energy content of plant rhizomes is important for plant survival under drought and heavy grazing stress, where large amounts of assimilates are translocated to the safe underground organs. This way, the chances for survival and continued vegetative growth are increased, particularly if the inflorescences and seeds are at risk of complete loss due to heavy grazing. The shift towards vegetative reproduction under drought and heavy grazing stresses indicates a strategy for longevity (Pianka, 1983). Under stress conditions, priority is given to the accumulation of more resources in the rhizomes than in the shoots where sexual reproduction has less priority. This is expected to give a high return on the energy investment required to produce new shoots around the year.

The judgment of rangelands in arid localities in the Middle East is usually based on an assessment of the vegetation and its component flora. This assessment can hardly be taken as an indicator of the pastoral value in the region. It is important to investigate the quantity, quality and seasonality of the grazable phytomass of the key species. This will help in estimating the amount of supplementary feed, determining the carrying capacity of different vegetation types, and setting rangeland management and conservation strategies.

It appears that the grazing value of the studied species goes hand in hand with the growth features and energy content in different localities of rangelands in arid regions of the Middle East. When the growth features and energy content are taken as a measure for the evaluation of plant grazability, the following sequence from high to low value of the species is obtained: *Panicum turgidum* > *Pennisetum divisum* = *Lasiurus scindicus*.

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EFFECT OF DIFFERENT DAMAGE FACTORS ON SOYBEAN SEED QUALITY

M. C. ROLLÁN[1], G. A. LORI[1,2], M. N. SISTERNA[1,2] and R. A. BARREYRO[3]

[1]CIDEFI (CENTRO DE INVESTIGACIONES DE FITOPATOLOGÍA)
[2]COMISIÓN DE INVESTIGACIONES CIENTÍFICAS DE LA PROVINCIA DE BUENOS AIRES, ARGENTINA
[3]DEPARTAMENTO DE PRODUCCIÓN VEGETAL, FACULTAD DE CIENCIAS AGRARIAS Y FORESTALES, UNIVERSIDAD NACIONAL DE LA PLATA, LA PLATA, BUENOS AIRES, ARGENTINA

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Poor quality in soybean seed can be due to physiological, pathological or mechanical causes. Seed morphological and anatomical features also make soybean more susceptible to damage factors than other plant species. The purpose of this paper was to evaluate the effects of the different damage factors upon soybean seed quality and its longevity during storage. In two trials, carried out in La Plata (Prov. Buenos Aires, Argentina), soybean seed samples from 7 pre-trading lines, obtained in two consecutive crops, were analysed. Assessments were performed on day 40 and day 160 after harvesting for the first trial and on day 50 and day 150 after harvest in the second. The method employed was the blotter test, following the International Seed Testing Association (ISTA) rules. The parameters evaluated were: damaged cotyledons, rotten seed, fungal contamination and germination capacity. The damage factors had different influences on seed quality. The presence of pathogenic fungi did not necessarily mean low germination capacity. Their effect depended on the degree of infection, the presence of seed-borne pathogens and the time of sample analysis. Rotten seeds and damaged cotyledons caused by moisture had a striking influence on seed quality and preservation during storage.

Key words: *Glycine max,* damage factors, moisture damage, seed-borne pathogens, seed quality, soybean seed, storage

Introduction

In the main soybean (*Glycine max* (L.) Merr.) seed production area in Argentina, the weather conditions during seed formation and desiccation up to the time of reaching commercial ripeness and harvesting tend to lead to the production of low seed quality.

The causal factors may act in either an additive or a synergistic manner, and may be of physiological, pathological or mechanical origin (Kueneman, 1981). According to Schultz and Francomano de Picardi (1989), these factors are, in order of importance: mechanical damage in wet harvested seed (over 14% humidity rate), plant pathogen, storage conditions, bugs, mechanical damage in dry harvested seed (under 12% humidity rate) and natural sudden drying in the plant and/or drier.

The anatomical and morphological features of soybean seeds make them more susceptible to damage factors than other plant species. Among these factors, the adverse effect of daily relative humidity (maximum and minimum values) during the physiological and commercial maturity and pre-harvesting stages causes a striking decrease in quality. During the above stages, the seed is exposed to constant hydration and dehydration processes to balance with the environment (Craviotto et al., 1989; França Neto et al., 1988). This behaviour is caused by the high permeability of its tegument (Hartwing and Potts, 1987). Potts et al. (1978) state that "impermeable tegument" or "hard seed" would be capable of preserving seed quality and delaying moisture absorption.

Soybean seed quality during storage is directly related to its previous history. Exposure to various factors may increase, preserve or decrease its quality (Krzyzanowski, 1989). Some of these factors cause irreversible damage, e.g. bug damage, unfavourable weather condition damage and mechanical damage, whereas the impact on quality of other types of damage, such as the presence of microorganisms, is variable. Several researchers (Athow and Laviolette, 1973; Dhingra et al., 1979; Jordan et al., 1988; Mitidieri, 1982) have studied the influence of pathogen fungi upon soybean seed quality, and found it to depend on the seed-borne pathogen, the degree of infection or even the moment at which the sample is analysed, since suitable storage conditions cause the inactivity of the microorganism (Sisterna and Lori, 1990).

The diverse factors affecting soybean seed quality may be of different importance for the preservation of viability during storage. Therefore, the aim of the present paper was to evaluate the influence that the different damage factors have upon seed quality and seed preservation during storage.

Materials and methods

The seed material used was provided by two trials, carried out in two consecutive years on the experimental fields of the Facultad de Ciencias Agrarias y Forestales (Universidad Nacional de La Plata) located in Los Hornos (first year) and in La Plata (second year).

The soybean seeds analysed comprised seven pre-commercial lines (L218, L221, L224, L227, L253, L300 and L1874) and the selections S5 and S6, from the breeding programme being carried out by the Plant Production Department at the same Faculty.

The lines completed their cycle between days 150 and 160 and were considered as maturity group V.

Sowing was carried out in November in both years. The plots consisted of 4 rows measuring 4.5 m with 20 plants/m, in a random block design with four replications. The trials were harvested 15 and 20 days after reaching commercial maturity in the two years, respectively. The seed was harvested with seed moisture contents of 13.7% and 12% for the two assays. During the first year, threshing was done mechanically, using an experimental thresher, whereas in the second year, in order to avoid mechanical damage, it was performed manually. Samples were kept under laboratory conditions (15°–20°C) in paper bags. This permitted the seed moisture contents to decrease during storage.

Analyses were carried out on day 40 and day 160 after harvesting in the first trial, and on day 50 and day 150 during the second trial.

On each date, 100 seeds from each line were analysed, discarding those that were split, atrophied or aborted due to the effect of *Nezara viridula* L. They were evaluated by the blotter test recommended by Neergaard (1974) and superficial disinfection was performed prior to sowing: 1) washing in running water for 10 minutes, 2) washing in 2% sodium hypochlorite (NaOCl) for 10 minutes, and 3) rinsing in distilled water.

The parameters determined were: a) Damaged cotyledons (%): germinated and non-germinated seeds having cuts or grooves with necrotic tissue both externally and internally; b) Rotten seed (%): those that did not germinate and had yellowish flaccid tissues; c) Fungally contaminated seeds (%): germinated and non-germinated seeds showing the presence of mycelium and/or fruit bodies. The microorganisms were isolated and cultured in 2% PDA for identification, with the purpose of studying their morphobiometric and cultural features; and d) Germination capacity (%): seeds with a radicle of over 2 cm were considered to be germinated.

Results

Prior to the analysis seed macroscopic observations were carried out and no significant morphological alterations were detected (1.4% aborted or atrophied discarded grains, 0.04% chalky grains, and 0.5% purple stained grains).

a) Damaged cotyledons: at the first date of analysis, a high percentage of damaged cotyledons was observed both in germinated and non-germinated seeds, in both seasons (Table 1). They were characterized by the presence of cuts and grooves in both cotyledons. These necrotic tissues, located in different areas, showed symmetrical injuries (Fig. 1). When the lesion appeared on the embryo axis or near it (vascular area), the germination process was interrupted and the radicle did not reach a length of 2 cm.

b) Rotten seed: in the first year values were higher than in the second one. Comparing the results of the two samplings this parameter showed substantial variations. As regards the two crops they were very low in case of recently harvested seeds, reaching extreme values of 49 and 11 at the end of the storage period (Table 1).

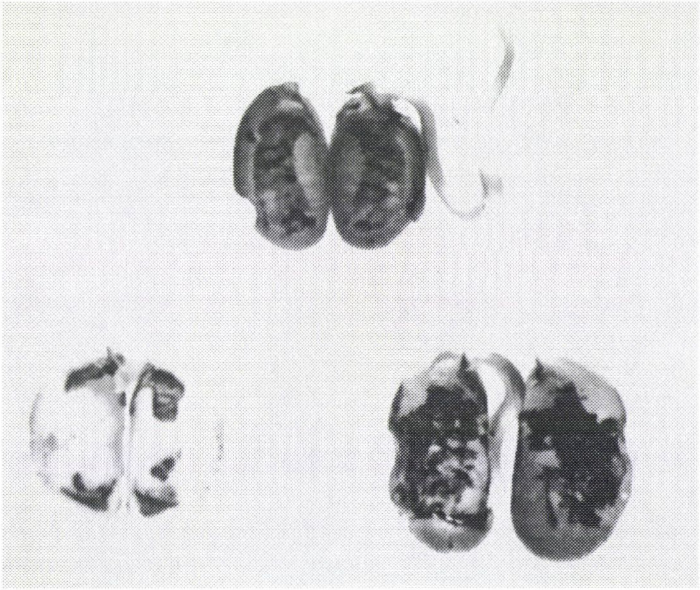

Fig. 1. Damaged cotyledons. Cuts and grooves placed symmetrically

Table 1
Parameters considered to evaluate soybean seed quality

Samples	1		2		3		4	
1st year	40 days	160 days	40 days	160 days	40 days	160 days	40 days	160 days
L 218	13/37	32/62	3	15	3/8	2/3	81	50
L 221	20/40	11/34	4	37	3/6	14/22	75	37
L 224	12/37	Nd	0	Nd	3/16	Nd	83	Nd
L 227	4/25	6/34	0	10	2/28	9/31	92	75
L 253	9/12	12/36	7	12	24/59	17/29	59	57
L 300	18/31	9/38	9	49	14/39	2/3	57	40
L 1874	Nd	26/60	Nd	4	Nd	0/5	Nd	57
2nd year	50 days	150 days	50 days	150 days	50 days	150 days	50 days	150 days
L 218	12/27	17/42	1	3	3/16	0/0	81	80
L 221	19/41	15/25	1	6	5/12	0/5	65	78
L 224	5/33	9/37	0	0	3/18	6/7	84	89
L 227	7/20	9/31	0	0	6/15	0/8	84	89
L 253	20/62	19/51	5	11	5/8	0/1	71	70
L 300	6/24	11/36	0	0	0/10	0/6	92	86
L 1874	5/12	23/46	0	0	3/23	1/10	91	75

1: Damaged Cotyledons (%); Non-germinated seed/Total seed; 2: Rotten Seed (%); 3: Fungal contamination (%) Non-germinated seed/Total seed; 4: Germination capacity (%); Nd: no data

The seed showed humidity rot, of a foetid odour, with yellowish, flaccid tissues, covered by mucous exudates of bacterial origin. Analysing the cause of these symptoms, it was clear that the seeds did not evidence alterations caused by insect stings or fungus presence. Instead, a great number of them had damaged cotyledons.

c) Fungally contaminated seed: the fungi observed in the analysed material were: *Phomopsis sojae* Leh, *Cescospora kikuchii* (Matsumoto and Tomoyasu) M. W. Gardner, *Fusarium* spp. (*F. graminearum* Schwabe, *F. pallidoroseum* Berk. & Rav. (=*F. semitectum*), *F. equiseti* (Corda) Sacc., *F. sambucinum* Fuckel var. *coeruleum* Wollenw., *F. solani* (Mart.) Sacc., *F. oxysporum* Schlecht) and *Alternaria* sp. Nees. (Table 2).

In the samples obtained from both assays, contaminated seed values showed variations along the analyses (Tables 1 and 2).

d) Germination capacity: this parameter was not stable during the storage period. In both years, the values detected on the first date of analysis (days 40 and 50 after harvest) were acceptable. On the other hand, there was a striking decrease as the storage period was prolonged in the first year. In the second year germination capacity remained fairly constant (Table 1).

Discussion

Each of the various damage factors to which soybean seed is exposed during the reproductive and pre-harvesting stages (bugs, pathogenic fungi and unfavourable environmental conditions) had a variable influence on seed quality.

Table 2
Mycoflora contaminating soybean seed at different dates of analysis in the 1st and 2nd year [Fungi (%)]

Samples	*Phomopsis sojae**		*Cercospora kikuchii**		*Fusarium* spp*		*Alternaria* sp.*	
1st year	40 days	160 days	40 days	160 days	40 days	160 days	40 days	160 days
L 218	0/0	0/0	2/7	1/1	0/0	0/0	1/1	1/2
L 221	0/0	0/0	1/3	1/1	0/0	2/2	2/3	11/19
L 224	0/0	Nd	0/4	Nd	1/1	Nd	2/11	Nd
L 227	0/0	0/0	0/0	0/2	0/2	0/2	2/26	9/27
L 253	0/2	0/0	0/1	1/2	1/1	3/5	23/55	13/22
L 300	0/0	0/0	0/2	1/1	2/3	0/0	12/34	1/2
L 1874	Nd	0/0	Nd	0/1	Nd	0/0	Nd	0/4
2nd year	50 days	150 days	50 days	150 days	50 days	150 days	50 days	150 days
L 218	3/5	0/0	0/9	0/0	0/0	0/0	0/2	0/0
L 221	2/2	0/0	2/8	0/5	0/0	0/0	1/2	0/0
L 224	1/2	4/4	2/14	0/3	0/0	0/0	0/2	2/2
L 227	3/6	0/0	1/3	0/5	0/0	0/0	2/6	0/3
L 253	0/1	0/0	2/3	0/1	0/0	0/0	3/4	0/0
L 300	0/2	0/0	0/6	0/0	0/0	0/0	0/2	0/6
L 1874	1/4	0/0	1/9	0/0	0/2	0/0	1/8	1/2

*Contaminated non-germinated seeds/total number of contaminated seed; Nd: no data

The data obtained in this assay showed that although different phytopathogenic genera were observed, their presence did not have an important effect on seed quality. A decrease in the fungus contamination during storage was also observed by Sisterna and Lori (1990), who demonstrated that the fungi found in the soybean seeds lost longevity. In many cases, contamination is limited to the external coats, due to the fact that infection occurs at the end of physiological ripeness. During phytosanitary tests, the constant contact of the cotyledons and the embryo axis with the infected tegument produced infected seeds or seedlings (França Neto and Henning, 1992). This was favoured more by the test conditions than by the pathogenic capacity of the organisms. This observation is contradicted by Jordan et al. (1988), Mitidieri (1982) and Ploper et al. (1989), who found that fungi were the main factors responsible for a decrease in seed quality and germination capacity.

In order to determine soybean seed quality it is recommended to combine the use of the tetrazolium, blotter and emergence in sand tests (França Neto and Henning, 1992). The blotter test technique allowed the identification of the fungi accompanying seeds and to carry out a follow-up through the successive dates of analyses, assessing their survival during storage and their effect upon germination capacity.

Cotyledons with cuts and grooves could be the consequence of mechanical damage caused by threshing, but it was determined in this assay that the cause of damage, in this case, was due to factors other than this. In the second year, in order to solve this problem, threshing was done manually.

According to Craviotto et al. (1989) and França Neto et al. (1988), during development and after reaching commercial ripeness soybean seed is exposed to constant dehydration and rehydration processes in the cells and tissues, with the aim of balancing their humidity with that of the atmosphere. During the developing stage or when already developed, the pressure exerted by the seminal teguments upon the tissues produces cuts on the cotyledons and the embryo axis of different degrees of intensity, known as humidity damage. This phenomenon was possible due to the fact that most of the soybean cultivars have a permeable tegument. This feature has been kept by the breeders, since it is desirable at germination time. However, it becomes a negative factor when the aim is to obtain good seed quality in areas where wide RH variations occur during the seed ripening season (Hartwing and Potts, 1987; Potts et al., 1978)

To corroborate that the samples analysed were exposed to such processes, daily maximal and minimal relative humidity (RH) values (%) were recorded during April and May, the months that correspond to stages R6, R7 and R8. According to these values, it could be observed that the material was exposed to great daily variations as regards environmental RH (Fig. 2).

Probably the higher values of rotten seed observed in the first year were due to the mechanical threshing, added to humidity damage. This last effect would cause cuts and grooves in the teguments and cotyledons that favoured gradual desiccation and loss of electrolytes till causing seed death (Craviotto et al., 1989; França Neto et al., 1988). They may also act as a means of entrance for contaminant bacteria that favour the rot symptom.

Damage caused by certain insects, such as sting bug *(Nezara viridula* L*).*, although irreversible, can be controlled. As regards pathogenic fungi, their presence does not necessarily mean quality decrease, since their influence on germination capacity is extremely low, since these fungi die during storage.

Moisture damage, reflected in damaged cotyledons and rot symptoms, is of great importance, having a notorious influence on germination capacity and seed viability during storage. Seed that suffers humidity damage has little or no chance of remaining viable till the next sowing. According to Dhingra et al. (1979) harvest delay produced bad seed quality. The results obtained showed that in spite of there being no great delay, the weather conditions predominant in the area at the time of harvesting were responsible for the damage.

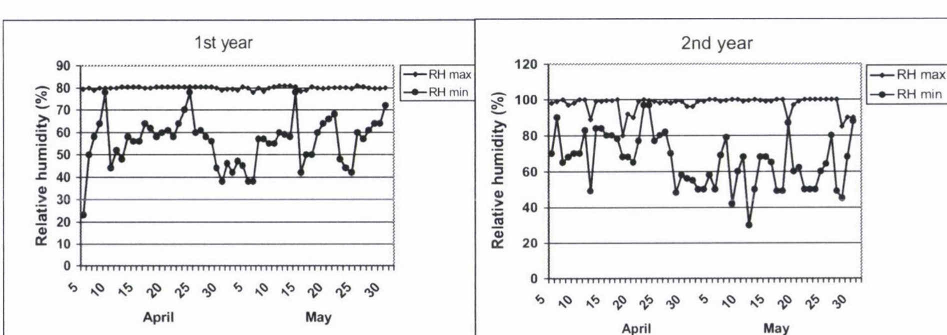

Fig. 2. Daily relative humidity, maximum and minimum values

In areas where weather conditions can cause this type of damage, shorter cycle cultivars or those with morphological features providing them with resistance against such weather conditions, should be chosen.

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EFFECT OF PHOSPHATE-SOLUBILIZING STRAINS OF *AZOTOBACTER CHROOCOCCUM* ON YIELD TRAITS AND THEIR SURVIVAL IN THE RHIZOSPHERE OF WHEAT GENOTYPES UNDER FIELD CONDITIONS

V. KUMAR[1], R. K. BEHL[2] and N. NARULA[1]

[1]DEPARTMENT OF MICROBIOLOGY, [2] DEPARTMENT OF PLANT BREEDING, CCS HARYANA AGRICULTURAL UNIVERSITY, HISAR, INDIA

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A field experiment was carried out to investigate the establishment of phosphate-dissolving strains of *Azotobacter chroococcum*, including soil isolates (wild type) and their mutants, in the rhizosphere and their effect on the growth attributes and root biomass of three genetically divergent wheat cultivars (*Triticum aestivum* L.). Four fertilizer doses were applied: 90 kg N ha^{-1}, 90 kg N + 60 kg P_2O_5 ha^{-1}, 120 kg N ha^{-1} and 120 kg N + 60 kg P_2O_5 ha^{-1}, besides a control plot without fertilizers or bioinoculants. Phosphate-solubilizing and phytohormone-producing parent soil isolates and mutant strains of *A. chroococcum* were isolated and selected following the enrichment method. On an overall basis the mutant strains performed better than the soil isolates for *in vitro* phosphate solubilization (11–14%) and growth hormone production (11.35%). Seed inoculation of wheat varieties with phosphate-solubilizing and phytohormone-producing *A. chroococcum* showed a better response over the control. Mutant strains of *A. chroococcum* showed a higher increase in grain (15.30%) and straw (15.10%) yield over the control and better survival (12–14%) in the rhizosphere as compared to their parent soil isolate (P4). Mutant strain M15 performed better in all three varieties in terms of increase in grain yield (20.8%) and root biomass (20.6%) over the control.

Key words: *Azotobacter chroococcum*, phytohormone, *Triticum aestivum*, phosphate solubilization, yield, root biomass, survival, phosphate-solubilizing bacteria (PSB)

Introduction

Generally the phosphate content in the soil is about 0.05% (w/w), but only a small fraction of the total soil phosphate is available to plants due to phosphate fixation by aluminium, calcium, iron, magnesium and soil colloids. Hence, the phosphate fertilizer efficiency is very low, particularly in calcareous (Khalafallah et al., 1982) and acid soil (Premono et al., 1996). Wheat varieties differ as regards nutrient use efficiency (El-Bassam et al., 1990) and recently CIMMYT has laid emphasis on the selection of high and low input use-efficient wheat genotypes for agro-climatic areas to maximize production. Moreover, integrated nutrient management strategies involving chemical fertilizers and biofertilizers have been suggested to enhance the sustainability of crop production (Manske et al., 1998). The role of microorganisms in phosphate

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solubilization or enhanced phosphate availability has been related to the production of organic acids, H_2S, mineral acids and to H^+ protonation (Kucey et al., 1989). Organic acids exuded by microorganisms form stable complexes with phosphorus adsorbents (aluminium, iron and calcium) and thus increase phosphate solubilization. Several studies have shown that as a soil bioinoculant *Azotobacter chroococcum* improved the growth of plants by various mechanisms like nitrogen fixation, ammonia excretion, growth hormone production, production of antifungal substances, siderophore and phosphate solubilization (Pandey and Kumar, 1989). However, studies on phosphate solubilization by *Azotobacter chroococcum* are meagre. Therefore, this study was conducted to investigate the establishment of phosphate-solubilizing strains, including soil isolates and their mutants, of *A. chroococcum* in the rhizosphere and their effects on plant and root growth attributes in genetically diverse wheat cultivars under field conditions.

Materials and methods

In vitro experiment

Phosphate (P)-solubilizing *A. chroococcum* was isolated from the wheat rhizosphere on the CCS Haryana Agricultural University farm in Hisar. Ten g of soil was inoculated in 100 ml of nitrogen-free Jensen medium (Jensen, 1951) amended with 2% tricalcium phosphate (TCP) and 1% Mussoorie rock phosphate (MRP) separately and were shaken at about 30°C for seven days. After five successive transfers P-solubilization was determined by measuring the zone of clearance (Premono et al., 1996) on Pikovskaya medium (Pikovaskaya, 1948) and Jensen medium containing 0.125% TCP, and also by the calorimetric method in liquid culture medium (John, 1970). Out of 164 P-solubilizing *A. chroococcum* soil isolates the five best were selected and designated as P1, P4, P9, P11 and P12, which solubilized 0.9496, 1.524, 1.139, 1.114 and 1.483 $\mu g\ ml^{-1}$ inorganic TCP and 0.1852, 0.1972, 0.1905, 0.1890 and 0.1924 $\mu g\ ml^{-1}$ MRP, respectively (Kumar and Narula, 1999). The indole acetic acid (IAA) production, as determined by the method of Tang and Bonner (1970), in the parent soil isolates was 174.6 μM (P1), 168.3 μM (P4), 139.5 μM (P9), 159.1 μM (P11) and 163.1 μM (P12). Out of these the best soil isolate, P4, which solubilized the maximum phosphate, was mutated with 50 $\mu g\ ml^{-1}$ of N-methyl-N-nitro-N-nitrosoguanidine (Adelberg et al., 1965) to obtain mutants having higher phosphate-solubilizing activity. N-methyl-N-nitro-N-nitrosoguanidine was dissolved in acetone and the parent isolate P4 cells were suspended in the mutagen at 30°C for 2 min, after which samples (0.1 ml cell suspension from mutagen solution) were plated on Jensen medium. The plates were incubated at 30°C. After 48 h clones were picked up and checked for P solubilization following the method of John (1970). Out of 256 mutants only 64 (25%) were able to solubilize more phosphate than the P4 parent isolate. Of these the five best were selected and designated as M14, M15, M22, M26 and M37, which solubilized 1.534, 1.776, 1.556, 1.598 and 1.745 TCP and 0.1997, 0.2272, 01975, 0.2238 and 0.2276 $\mu g\ ml^{-1}$ MRP, respectively (Kumar and Narula, 1999). IAA production in these mutants was maximum in M14 and M26 (184.5 μM), followed by M22 (184.0 μM), M15 (181.1 μM) and M37 (179.2 μM) (Kumar and Narula, 1999). For seed treatment all the ten bacterial strains of *A. chroococcum* were grown in Jensen medium at 30°C with constant shaking for four days till a population of 10^9 cells ml^{-1} was attained.

Field experiment

For the field experiment pre-inoculated seeds of three wheat (*Triticum aestivum* L.) varieties, C-306 (low P responsive), HD-2009 (medium P responsive) and WH-542 (high P responsive), were sown in sandy loam alluvial soil having pH 8.5, organic carbon 0.35%, total nitrogen 0.034% and available phosphate 4.2 ppm. Fertilizer was applied at the rate of 90 kg N ha^{-1}, 90 kg N + 60 kg P_2O_5 ha^{-1}, 120 kg N ha^{-1}, and 120 kg N + 60 kg P_2O_5 ha^{-1} besides a control plot (without fertilizer or bioinoculants). Observations on grain and straw yields (kg ha^{-1}), plant height (cm), dry matter (g m^{-1} row length), grain weight $spike^{-1}$, grains $spike^{-1}$, 1000 grain weight (g) and root biomass (mg $plant^{-1}$) were recorded at the time of harvest (120 days after sowing). The survival of inoculated bacteria was observed on the 20^{th}, 40^{th}, 60^{th} and 80^{th} days after sowing. The rhizospheric soil, adhering intimately to the roots, was separated by gentle tapping and composite samples were prepared. The soil samples were then air dried at room temperature and the bacterial count of inoculated *A. chroococcum* was determined by the dilution plate technique. The marker methyl ammonium chloride was used in the medium to count only inoculated *A. chroococcum* (Bela et al., 1986). The data were analysed at the 5% level of significance (Cochran and Cox, 1967).

Results

In general, all the quantitative plant traits and root biomass showed a significant increase which was almost linear in response to chemical fertilizer treatments, the maximum being at 120 kg N + 60 kg P_2O_5 ha^{-1}. Cultivar C 306 exhibited maximum plant height and 1000 grain weight, while WH 542 excelled in grain and biological yield, grains $spike^{-1}$ and dry biomass. The varieties did not differ for root biomass. This response was further substantiated with bacterial inoculation (Table 1). The combined effect of bioinoculants (parent isolates and mutant strains) and fertilizers showed maximum grain yield in the high P-responsive semi-dwarf wheat variety WH 542 (2832 kg ha^{-1}), while the straw yield was maximum in HD 2000 (4930 kg ha^{-1}). All the bioinoculants performed better than the control and exhibited a significant increase in grain (10.3%) and straw (11%) yield, while mutant strains of *A. chroococcum,* particularly M15, showed the highest increase in grain yield (20.8%) over the control and even over its parent isolate (14.7% more). Averaged over the fertilizer treatments and cultivars, the grain and straw yield was higher for the mutants (10.4% and 10.1%, respectively) as compared to the parent isolates. Among the mutant strains, M15 showed higher grain (20.8%) and straw yield (20.6%), dry weight (19.8%), grains $spike^{-1}$ (23.06%) and 1000 grain weight (14.36%) than the control (Table 1). The mutant strain M 37 produced higher plant height (22.7%) and grain weight $spike^{-1}$ (40.5%) (Table 2). Root biomass was significantly higher in mutants (8.48%) and parents (2.71%) as compared to that of the control (Tables 1 and 2). It is evident from the data summarized in Table 3 that the bacterial count increased gradually and reached a maximum on the 80^{th} day in the parent isolates and mutant strains. In general the survival of the mutants in the rhizosphere was considerably higher (12–14%) than that of the parent isolates in all the fertilizer treatments and cultivars. The rhizosphere of WH 542, in general, showed 10.9 to 14.7% higher bacterial counts of the mutant strains than of the parent isolates.

Table 1
Effect of fertility levels, wheat varieties and *Azotobacter chroococcum* inoculants on yield parameters of wheat genotypes under field conditions

Treatments	GY*	SY	DM	GS	1000 GW
Fertility levels					
Control	1294	2331	82.5	51.4	35.7
90 N (kg ha^{-1})	2529	4565	159.6	55.9	37.1
90 N + 60 P_2O_5 (kg ha^{-1})	2742	5005	172.6	60.5	38.3
120 N (kg ha^{-1})	3185	5681	195.1	61.4	38.5
120 N + 60 P_2O_5 (kg ha^{-1})	3405	6088	212.6	62.6	38.4
SEm ±	687	106	4.08	1.9	0.7
CD at 5%	206	321	12.37	5.8	2.0
Varieties					
C 306	2309	4912	159.7	51.9	41.2
WH 542	2832	4892	168.6	69.3	35.7
HD 2009	2752	4930	168.0	53.8	36.8
SEm ±	53	82	3.08	0.4	0.5
CD at 5%	160	248	9.34	1.1	1.5
A. chroococcum inoculants					
Control	2398	4304	149.5	49.0	34.8
P 1	2483	4502	157.2	53.4	38.6
P 4	2619	4703	167.7	54.8	38.4
P 9	2527	4569	159.5	53.7	38.2
P 11	2446	4594	157.3	53.1	38.5
P 12	2561	4630	158.1	54.3	38.3
M 14	2661	4777	168.4	55.7	37.8
M 15	2899	5193	179.1	51.3	38.6
M 22	2730	4872	171.8	55.8	38.3
M 26	2734	4904	173.1	55.7	37.8
M 37	2802	5027	174.3	60.3	39.8
SEm ±	64	84	2.25	2.8	0.8
CD at 5%	177	233	7.06	7.7	2.3
Control vs. parents (wild type) and mutants of *A. chroococcum*					
Control	2398	4304	149.5	49.0	34.8
Parents	2527	4599	159.9	53.8	38.4
Mutants	2765	4954	170.3	55.7	38.4
SEm ±	47	62	1.6	2.0	0.6
CD at 5%	130	172	4.4	5.5	1.6

*GY: grain yield (kg ha^{-1}); SY: straw yield (kg ha^{-1}); DM: dry matter (g/m row length); GS: grains/spike; 1000 GW: 1000 grain weight (g)

Table 2

Effect of fertility levels, wheat varieties and *Azotobacter chroococcum* on biological yield (kg ha^{-1}), plant height (cm) and root biomass (mg plant $^{-1}$) of wheat genotypes under field conditions

Treatments	Biological yield	Plant height	Root biomass
Fertility levels			
Control	3625	67.20	991
90 N (kg ha^{-1})	7094	69.42	1191
90 N + 60 P_2O_5 (kg ha^{-1})	7747	75.60	1301
120 N (kg ha^{-1})	8866	77.71	1330
120 N + 60 P_2O_5 (kg ha^{-1})	9493	82.30	1531
SEm ±	126	1.81	31
CD at 5%	382	5.49	94
Varieties			
C 306	7221	83.90	1231
WH 542	7724	72.31	1244
HD 2009	7684	67.21	1259
SEm ±	97	1.28	25
CD at 5%	294	3.88	NS
A. chroococcum inoculants			
Control	6686	65.20	1179
P 1	6985	73.70	1152
P 4	7322	70.71	1238
P 9	7096	72.11	1215
P 11	7040	76.20	1231
P 12	7191	69.90	1214
M 14	7438	78.80	1242
M 15	8092	80.00	1278
M 22	7602	79.00	1265
M 26	7638	76.20	1269
M 37	7829	75.50	1342
SEm ±	105	0.55	23
CD at 5%	291	1.99	64
Control vs. parents (wild type) and mutants of *A. chroococcum*			
Control	6686	65.20	1179
Parents	7126	72.52	1211
Mutants	7719	77.90	1279
SEm ±	78	0.40	16
CD at 5%	216	1.11	44

Discussion

The response of the wheat crop to inorganic fertilizer is well understood; however, a combination of microbial inoculants and fertilizer has shown variable results. Genetic variability exists among wheat genotypes for nutrient use and production *per se* (Manske et al., 1998). In the present study it was evident that the increase in fertilizer doses manifested a significant and almost linear increase in grain yield and root biomass.

Table 3

Population of *Azotobacter chroococcum* strains (wild type and mutants) in the rhizosphere of wheat plants under field conditions

Varieties	P1	P4	P 9	P 11	P12
Observation on 20th day					
C 306	2.5×10^3	2.6×10^3	2.6×10^3	2.8×10^3	3.0×10^3
WH 542	2.6×10^3	2.7×10^3	2.9×10^3	2.9×10^3	3.0×10^3
HD 2009	2.7×10^3	2.8×10^3	2.7×10^3	2.8×10^3	2.9×10^3
Mean	2.6×10^3	2.7×10^3	2.7×10^3	2.8×10^3	3.0×10^3
Observation on 40th day					
C 306	3.1×10^3	3.1×10^3	3.0×10^3	2.8×10^3	2.9×10^3
WH 542	3.1×10^3	3.0×10^3	3.0×10^3	3.1×10^3	3.1×10^3
HD 2009	3.2×10^3	3.1×10^3	3.2×10^3	3.0×10^3	3.2×10^3
Mean	3.1×10^3	3.1×10^3	3.1×10^3	3.0×10^3	3.1×10^3
Observation on 60th day					
C 306	2.8×10^5	2.9×10^5	3.0×10^5	3.1×10^5	2.7×10^5
WH 542	3.1×10^5	3.1×10^5	2.9×10^5	3.4×10^5	2.9×10^5
HD 2009	2.9×10^5	3.1×10^5	2.9×10^5	3.3×10^5	3.1×10^5
Mean	2.9×10^5	3.0×10^5	2.9×10^5	3.2×10^5	2.9×10^5
Observation on 80th day					
C 306	3.3×10^4	3.2×10^4	3.3×10^4	3.4×10^4	3.1×10^4
WH 542	3.3×10^4	3.1×10^4	3.4×10^4	3.2×10^4	3.0×10^4
HD 2009	3.4×10^4	3.3×10^4	3.6×10^4	3.7×10^4	3.1×10^4
Mean	3.3×10^4	3.1×10^4	3.4×10^4	3.4×10^4	3.1×10^4
	M14	M15	M 22	M26	M37
Observation on 20th day					
C 306	3.3×10^3	3.0×10^3	3.0×10^3	2.8×10^3	3.2×10^3
WH 542	2.9×10^3	2.9×10^3	3.2×10^3	3.0×10^3	3.2×10^3
HD 2009	3.0×10^3	3.0×10^3	3.2×10^3	3.0×10^3	3.2×10^3
Mean	3.1×10^3	3.0×10^3	3.2×10^3	3.0×10^3	3.2×10^3
Observation on 40th day					
C 306	3.6×10^3	3.6×10^3	3.4×10^3	3.5×10^3	3.5×10^3
WH 542	3.5×10^3	3.5×10^3	3.5×10^3	3.4×10^3	3.6×10^3
HD 2009	3.7×10^3	3.6×10^3	3.3×10^3	3.7×10^3	3.3×10^3
Mean	3.6×10^3	3.6×10^3	3.4×10^3	3.5×10^3	3.5×10^3
Observation on 60th day					
C 306	3.0×10^5	3.1×10^5	3.1×10^5	3.1×10^5	3.4×10^5
WH 542	3.4×10^5	3.4×10^5	3.4×10^5	3.1×10^5	3.3×10^5
HD 2009	3.3×10^5	3.4×10^5	3.2×10^5	3.5×10^5	3.5×10^5
Mean	3.2×10^5	3.3×10^5	3.2×10^5	3.3×10^5	3.4×10^5
Observation on 80th day					
C 306	3.6×10^4	3.5×10^4	3.7×10^4	3.4×10^4	3.7×10^4
WH 542	3.6×10^4	3.8×10^4	3.8×10^4	3.5×10^4	3.8×10^4
HD 2009	3.8×10^4	4.0×10^4	3.8×10^4	3.9×10^4	4.0×10^4
Mean	3.6×10^4	3.8×10^4	3.8×10^4	3.6×10^4	3.8×10^4

The high input wheat variety WH 542 performed significantly better than the other varieties over all the fertilizer treatments for grain and most other characters. The rhizosphere of cereal crops was found to harbour a great number of PSB (phosphate-solubilizing bacteria) isolates (Leinhos and Bergmann, 1995). The productiveness of the rhizosphere for PSB may be attributed to the favourable influence exerted by root exudates (Vancura and Harizlikova, 1972; Leinhos and Vacek, 1994), which contain amino acids, carbohydrates, organic acids and growth-promoting substances. The activity of phosphate-solubilizing and growth hormone-producing *A. chroococcum* exerted a considerable influence on plant nutrient uptake and root biomass (Barea et al., 1976; Kundu and Gaur, 1980).

The comparative evaluation of parent soil isolates P1, P4, P9, P11 and P12 and mutants M14, M15, M22, M26 and M37 in the present studies revealed that the mutants, on average, solubilized more TCP (13.21%) and MRP (14.05%). Also, hormone production in the mutants was about 11.35% higher than in the parent soil isolates. When compared with parent soil isolate P4, the mutants solubilized 11.4 (M37) to 13.2% (M14) more TCP and 11.3 (M22) to 14% (M14) more MRP, while producing 10.64% (M37) to 10.96% (M15) more IAA. Consequently, the inoculation of phosphate-solubilizing and phytohormone-producing *A. chroococcum* mutants exerted a considerably more favourable influence on wheat, as shown by increased grain, straw, biological yield, dry matter, plant height, root biomass, grains spike^{-1} and 1000 grain weight over the control. Thus, the application of mutant strains of *A. chroococcum* in the present studies may have led to more solubilization of insoluble phosphate in the soil and consequently higher uptake of phosphate. This was further substantiated by the observation that available P_2O_5 decreased towards the maturation of the crop in the pot soil. Also, the greater uptake of phosphorus in the same wheat cultivar using the same bioinoculants was observed under field conditions (Kumar, 1998). Seed or soil inoculation with phosphobacteria was reported to improve the root biomass of various crops (Khalafallah et al., 1982). The increased plant growth parameters and root biomass in the case of mutant strains could be attributed to the production of a higher quantity of growth-promoting substances and the complementary effect of enhanced phosphate availability, as reported earlier by Gaind and Gaur (1991). Although the wheat varieties did not differ significantly for root biomass, the different soil isolates and mutants exhibited differences in survival rate (bacterial) in the rhizosphere of different varieties. This indicated that the qualitative and quantitative aspects of root exudates had a greater influence than root biomass *per se*. Moreover, as compared to soil isolates PSB mutants maintained their higher number (12–13%) in the inoculated treatment throughout the growth period of the crop, the highest being in the case of M15 on the 60th day (14.7% higher than P4) in the rhizosphere of the high P responsive cultivar WH 542. The stimulatory effect of the chemical fertilizers on the survival of *A. chroococcum* may be exerted directly through their effect on the growth and proliferation of the bacterium itself or indirectly by changing the growth rate and metabolic activities of the crop plants, resulting in more root exudates and thereby creating a favourable habitat for the growth and development of these microorganisms (Gaind and Gaur, 1991). Microbial inoculation, in the form of P-solubilizing, phytohormone-producing *A. chroococcum,* may also augment the efficiency of applied and native P_2O_5 by reducing phosphate fixation in the soil fractions. This study suggests that (a) it is possible to select physiologically efficient strains of *A. chroococcum* through mutagenesis using soil isolates and (b) microbial inoculants can be used as an economic input to increase crop productivity at lower fertilizer levels with the uptake of more nutrients from the soil.

Conclusions

It is clear from the results that an integrated nutrient management strategy should be adopted for sustainable land use and higher production. Microorganisms may play a vital role in reducing the amount of chemical fertilizer in agriculture and also in mobilizing fixed phosphate fertilizers in the soil.

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SOIL PRODUCTIVITY ASSESSMENT METHOD FOR INTEGRATED LAND EVALUATION OF HUNGARIAN CROPLANDS

G. TÓTH

GEORGIKON FACULTY OF AGRICULTURE, UNIVERSITY OF VESZPRÉM, KESZTHELY, HUNGARY

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Land productivity evaluation systems are developed to predict the crop growing potential of lands on the basis of their attributes. The Hungarian land evaluation system presently in use, known as the gold crown system, was developed in the 19th century and its rating is based on profitability. This system does not give an exact description of the productivity potential of the land and includes no information on the environmental characteristics of the soils. In recent decades most European countries have adopted land evaluation methods based on land and soil parameters. In the 1980s a quantitative land evaluation method of this type was introduced in Hungary as well. In this system the effect of soil attributes on the level of fertility was expressed in numerical terms by soil mapping units. This system was again replaced by the gold crown system during the political changes in the early 90s. However, using a soil evaluation system (where the relative production potential of the land is expressed in a quantitative manner) together with measurements of soil degradation or amelioration an integrated method could be developed to express various land quality/land productivity relationships. This approach could help decision makers – along with land users and environmental scientists – to choose profitable and sustainable land use types and methods at local and regional levels. In this context, sustainable land use means biomass production with the highest efficiency without harmful environmental side effects.

This paper introduces a soil evaluation methodology based on the Hungarian genetic soil classification. The productivity evaluation system was worked out on the basis of long-term (6 years) yield data collected from 1019 fields in the Balaton Upland region (Central Western Hungary) and large-scale genetic soil maps of the study region.

Key words: land evaluation, soil productivity, productivity potential, soil quality

Introduction

Crop production is a process which converts solar energy into biomass. In order to increase the efficiency of this process, land users apply additional inputs, such as fertilizers, irrigation, etc. In other cases, income is generated by extending cultivation to areas under environmental risk. Economic factors play a crucial role in the decisions made by farmers on the allocation of production and input intensity. Sustainable land use, however, requires the inclusion of ecological criteria in planning and management practices. Ecological site assessment and land productivity evaluation are among the most comprehensive tools available to support land use planning.

Traditional land evaluation systems, such as the century-old Hungarian 'gold crown' system, are based on profitability evaluation and do not take the ecology of the land into account. These systems are controversial indicators of

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productivity and unable to fulfil the land evaluation needs of a modern society. Two of the basic requirements for an up-to-date evaluation system are that it should be plant-specific and based on natural conditions. Apart from their crop growing potential, other ecological functions of the soil also have increasing importance in sustaining ecological and economic (thus social) systems. This aspect should also be included in a complex land evaluation system.

One of the approaches used to describe the multifunctional nature of the soil is the soil quality concept (Doran and Parkin, 1996; Karlen et al., 1997). According to the most widespread interpretation of this concept, there are three major components of soil quality, namely: 1) sustainable productivity, 2) environmental quality, 3) plant and animal health (Mausbach and Tugel, 1997; Michéli, 1999). The integration of these aspects in future land evaluation systems could help to achieve complex natural resources management goals. The question arises of how this integration could be carried out and what common basis could be employed to express these characteristics.

Since on the one hand major soil-forming factors are among the most influential conditions of production potential, while on the other hand, the genetics of the soil decisively determines its ecological responses an approach based on genetic soil classification could provide one of the most comprehensive answers. Other important arguments for this kind of approach are soil taxonomic classification and mapping, both of which are based on the genetic characteristics of the soil. Soil taxonomy categorizes soils on the basis of measurable attributes, while soil mapping helps in the spatial presentation of soil information.

The exact description of soil quality (whether the soil function considered is fertility or something else) is a matter of taxonomic details. However, the capacity to function can be expressed in relative terms within the classification framework. It is necessary to assess the role of soil attributes at the level of fertility and of different kinds of environmental quality. If these assessments are carried out for genetic soil units, common ground can be found for various types of comparative analyses.

The specification of soil attributes and of the interrelation between the attributes that are the most important for the fertility level needs a complex approach in itself. This approach could be based on genetic soil classification, as this type of soil classification makes it possible to describe not only the nature of soil attributes but also the soil productivity characteristics (water and nutrient dynamics) of agricultural land.

Soil genetics also provides a good basis for estimating the ecological behaviour of the soil. The loss in soil productivity due to various kinds of soil degradation is an inherent characteristic of different soil units. The quantitative expression of the land's relative production potential combined with the measurement of various kinds of soil degradation (erosion, acidification, compaction, etc.) provides an integrated method for the expression of land quality/land productivity relationships.

During the productivity evaluation process, various soil attributes (texture, humus content, thickness of humus layer, pH, parent material, etc.) can be characterized by numerical values (correction factors) according to their relative importance in the production potential of different genetic soil subtypes. A standard fertility index can be set for each genetic soil subtype, corresponding to the relative fertility of the most common variety in the subtype. By applying the above correction factors according to the genetic subtype of the soil variety, the actual relative fertility of the soil variety can be characterized quantitatively. Fórizs et al. (1972), Sisov et al. (1991), Van den Born and Vogel (1986), Vlad et al. (1996) and Wu (1993) used similar approaches in their productivity evaluation systems.

The yield response to fertilizer (and irrigation or other amelioration practices) is a distinctive characteristic of genetic soil varieties. If productivity ranking and fertilizer (or other inputs) response information are available, land use planners have a complete set of information for putting production on a sound economic basis.

To match economic and environmental management goals, soil quality and degradation risks have to be assessed as well. To a great extent the risk of degradation is a soil type-dependent phenomenon. Therefore, when assessing the possible impact of environmental or anthropogenic factors on land use, a soil type-based approach to risk assessment should be applied. By integrating the results of land productivity and environmental quality assessments, sufficient information can be obtained for purely economic cost-benefit evaluations, as well as for environmental impact characterization. Thus, energy efficiency evaluation can be compared with ecological criteria.

Materials and methods

The cultivated fields of the Balaton Upland region in Central Western Hungary were selected as sample fields for model development. Landform, soil type, land management and crop yield information was collected from 1019 fields, covering 26,921 ha of land. Detailed 1:10,000 scale soil maps are available for 15,161 ha of this cropland. The *MS Access* relational database manager was used to input, organize and store primary data. The fertilization and crop production data of 6 consecutive years (1984-1989) were collected and processed. Seven major soil types (or subtypes) occupy 73.13 % of the whole area. These are the following: Ramann's brown forest soil on sand (or rusty brown forest soil, Arenic Eutrochrept); carbonated meadow soil (Typic Endoaquoll); humus carbonate soil (Typic Udorthent); brown rendzina soil (Lithic Rendoll); lessivated brown forest soil (Typic Haplustalf); Ramann's brown forest soil (Typic Eutrochrept) and colluvial soil (Typic Udifluvent). The latter five soils were the most widespread on the mapped fields, covering 70.85% of the mapped area. The names in parenthesis correspond to the analogous soil units in the US Soil Taxonomy (Buol et al., 1989; Soil Survey Staff, 1990; 1996).

In the first phase of the productivity evaluation, box-plot analyses were carried out to match wheat and maize yield data with the genetic soil types and subtypes of the sample fields. Using this method, the main characteristics of different soil types can be described and they can be ranked in terms of relative fertility. The geographical distribution of fields of different size and soil type showed great variation. To avoid overestimating the fertility of any soil type because of some 'climatic year effect'[1] (or other distinct influence) in a certain location, the calculations were based

[1] Climatic year effect is an integrated index (including annual precipitation, temporal distribution of rainfall, heat sum, etc.) which can be defined as the meteorological factor influencing plant production over the period of the production year.

on area measurements weighted by the square roots of the size in hectares. The ANOVA method and the Tukey test were used to verify the assumptions drawn from the results of the box-plot analyses. Wheat was used as indicator crop in these and in all the later analyses.

In order to reduce the yield variability originating from 'climatic year effects' and the effect of fertilization, the yield data were weighted. Regression analyses were carried out to identify the amount of fertilizer-induced yield increase on different soil subtypes. The effect of fertilization was investigated for the five major soil types separately. A similar method was used to measure the 'climatic year effect'.

In the next phase of the analysis, the productivity of soil units at lower levels of the taxonomic classification was assessed. Regression analyses were used to assess the influence of different soil attributes (which are also the bases of genetic soil variety classification) on the level of fertility of different genetic soil types (taken from soil maps).

Results and discussion

Box-plot analyses indicate the differences between the productivity of genetic soil types/subtypes (Figs. 1, 2, 3). ANOVA and the Tukey test verified these differences, and provided a positive answer to the question of whether genetic soil (sub)type was a yield-differentiating factor. The mean difference in wheat yields was found to be significant across soil types (and subtypes) on a long-term basis (Table 1). The order of productivity level (and yield variability) for different soil units in the case of wheat and maize showed certain variation (Figs. 1, 3), indicating that the relative crop productivity of the soil is a plant-dependent characteristic.

Although some of the soil types were not significantly different from each other in long-term fertility, the overall range of the mean levels of long-term yields – even in the case of closely related soil types – supported the hypothesis that there are differences between the crop-growing potential of different genetic soil types. In the pairwise comparison, 14 out of 22 pairs of soil types/subtypes showed significant differences in the yield levels (Table 2), underlining the fact that in most cases the genetic soil subtype could be a yield-differentiating factor.

The facts outlined above obviously do not mean that a knowledge of the genetic soil type or subtype of two soils is sufficient to tell which is more productive. For exact productivity identification it is necessary to explore the relationship between various clusters of soil characteristics (as well as individual soil attributes) and fertility. In other words, it is necessary to extend the analyses to lower taxonomic levels of the genetic soil classification system.

For a more exact analysis of the influence of soil and land characteristics on yield levels, it is necessary to consider the effect of fertilization and other yield-increasing management factors as well. By incorporating the effect of fertilization, the land evaluation model can be made to express an element of intensive, semi-intensive or extensive land management that is important both from the economic and the ecological point of view. Since fertilization contributes over 30% of the total energy demand of intensive crop production, most of which comes from N fertilizers, it is very important to analyse fertilizer efficiency within the framework of productivity evaluation.

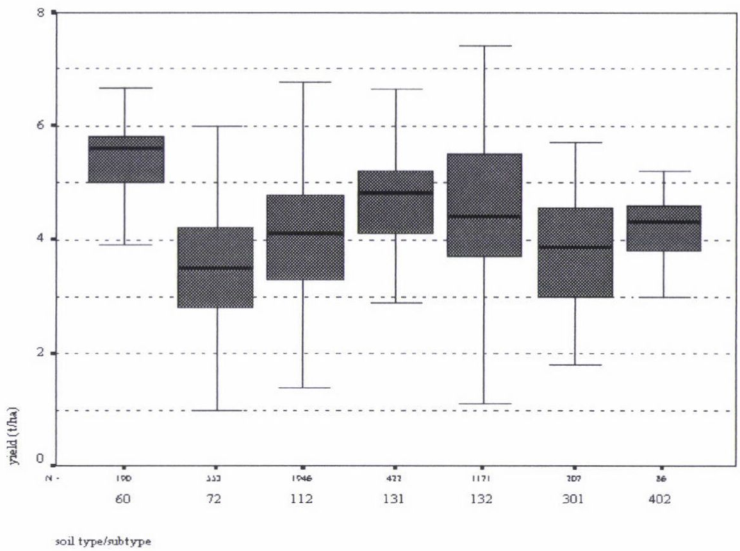

Fig. 1. Long-term wheat yield levels of some selected soils. Based on the aggregation of six years (1984–1989) of data (60: Humus carbonate soil; 72: Brown rendzina soil; 112: Lessivated brown forest soil; 132: Ramann's brown forest soil; 132: Rusty brown forest soil; 301: Carbonated meadow soil; 402: Colluvial soil)

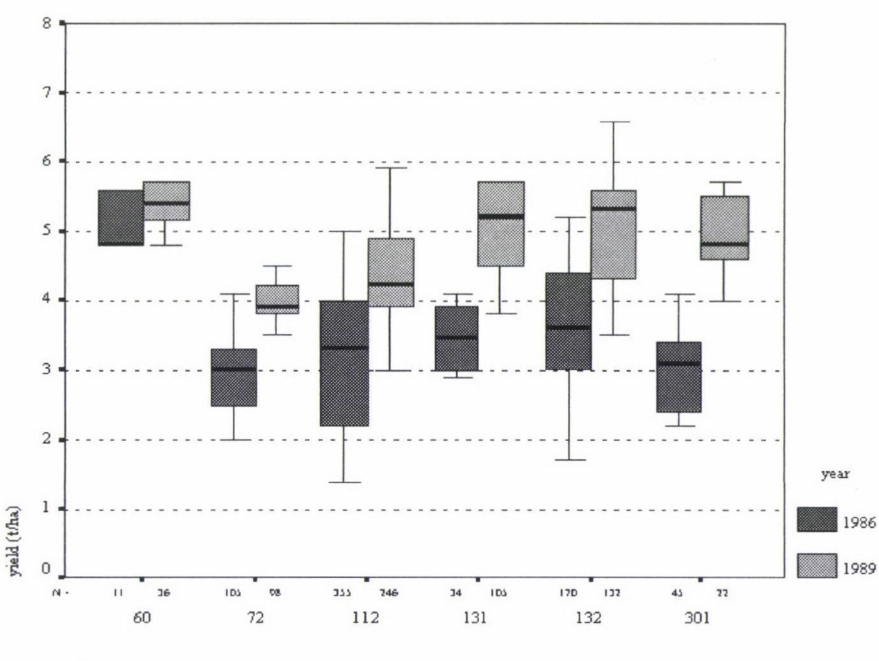

Fig. 2. Wheat yield levels of some selected soil (sub)types. For legend, see Fig. 1

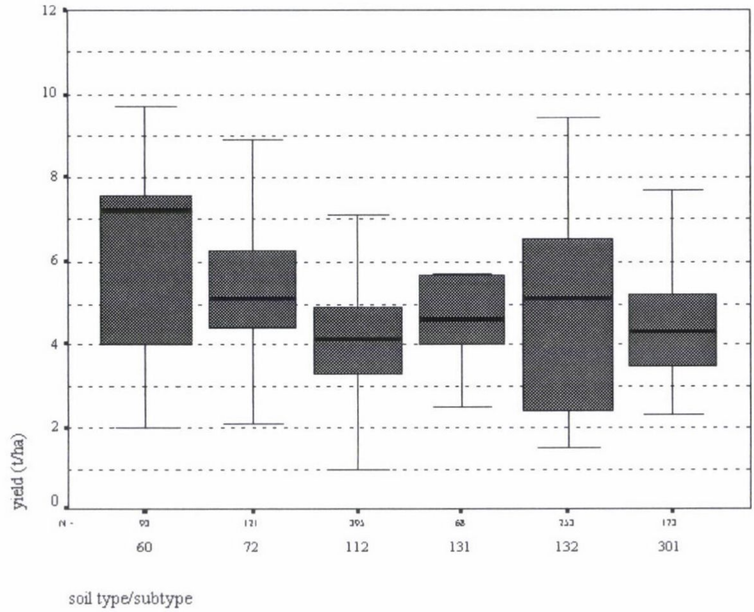

Fig. 3. Long-term maize yield levels of some selected soils. Based on the aggregation of six years (1984–1989) of data. For legend, see Fig. 1

Table 1
Analysis of variance of the effect of soil type on wheat yield

	Sum of squares	df	Mean square	F	Sig.
Between groups	139.430	6	23.238	19.321	0.000
Within groups	1037.971	863	1.203		
Total	1177.402	869			

The analysis of fertilizer response showed the following correlation when no distinction was made between the genetic soil types[2]:

$Y=2.724 + 0.0109N+0.0029P$ ($R^2=0.198$; $p=0.000$; sig.N: $p=0.000$; sig.P: $p=0.070$)

where Y = t/ha wheat yield; N = kg/ha nitrogen fertilizer and P = kg/ha P_2O_5 fertilizer.

The same analysis for brown rendzina soils resulted in the function:

$Y = 2.353 + 0.010N + 0.006P$ ($R^2=0.190$; $p=0.000$);

[2] Exponential functions would be more appropriate to quantify the effect of fertilizer; however, with the given amount of data no significant result was found when the analysis was carried out for the different soil (sub)types separately. Nevertheless, linear correlations can be used to describe the soil evaluation approach.

for lessivated brown forest soil:

$$Y=3.151+0.0075N+0.0018P\ (R^2=0.238;\ p=0.003);$$

for Ramann's type brown forest soil:

$$Y=3.778+0.0083N-0.0049P\ (R^2=0.187;\ p=0.009).$$

For humus carbonate soils and colluvial soils no significant functions could be determined (presumably due to the small sample size), so the first equation was used to calculate the effect of fertilization on these soils.

To identify the attributes (and/or clusters of attributes) within the genetic soil units that are the most important determinants of fertility level and to describe the soil-attribute-driven regulatory principles of water and nutrient availability and dynamics in soils, detailed soil evaluation analysis was carried out using multiple regression analysis based on genetic soil units. Partial correlation coefficients were derived for each soil attribute investigated, indicating their influence on the level of fertility.

As a result of the iterative analyses, following the series of regression analysis weighting factors were assigned to each investigated soil characteristic based on genetic soil types. The weighting factors are based on the partial correlation coefficients of the regression analyses and express the relative importance of the characteristics in the fertility level of the genetic soil unit. These weights (or factors) are the control parameters of the soil evaluation model. If the genetic soil type (subtype), and thus the standard fertility index, is known, these factors can be used to evaluate the productivity of the investigated field or plot. Table 3 presents the control parameters of some selected soil attributes for Typic brown forest soils.

Table 2

Relations between the t/ha yields of the different soil (sub)types (Results of the Tukey test)

Soil types (subtypes)	CS[+]	CMS	RBFS	RaBFS	LBFS	BRS
CMS	–0.6360					
	(0.257)					
RBFS	0.0120	0.6238**				
	(0.225)	(0.166)				
RaBFS	0.3325	0.9658**	0.3447			
	(0.246)	(0.194)	(0.149)			
LBFS	–0.3403	0.2958	– 0.3281*	– 0.6728**		
	(0.218)	(0.157)	(0.096)	(0.139)		
BRS	–0.7857*	–0.1496	– 0.7735**	– 1.1182**	– 0.4454**	
	(0.235)	(0.179)	(0.123)	(0.164)	(0.118)	
HCS	1.1474**	1.7835**	1.1596**	0.8149*	1.4877**	1.9931**
	(0.290)	(0.248)	(0.215)	(0.237)	(0.208)	(0.225)

[+]CS: Colluvial soil; CMS: Carbonated meadow soil; RBFS: Rusty brown forest soil; RaBFS: Ramann's brown forest soil; LBFS: Lessivated brown forest soil; BRS: Brown rendzina soil; HCS: Humus-carbonate soil; *,**: Mean differences significant at the 0.05 and 0.01 levels, respectively; Figures in parentheses are standard errors of the means

Table 3
Effect of soil attributes on the fertility of Typic brown forest soils

Parent material	Weight factor	Texture	Weight factor	Humus content	Weight factor
Sand	0.75	Sandy loam	0.85	Low	0.95
Loessy sand	1.15	Loam	1.15	Medium	1.05
Double layered loess	0.8	Clayey loam	1.05	High	1.15
Silty loess	1.0	Clay	0.92		
Loessy clay	1.0				
Marly clay	1.5				

By including more variables in the evaluation process, the soil evaluation method described above could be an appropriate tool for establishing a cohesive productivity classification of agricultural fields with different ecological characteristics.

The soil evaluation system should be structured in such a way that different aspects of soil quality could be expressed within the same classification framework, in a clear and comprehensive manner.

The "base fertility" of the soils is graded on a 1 to 100 scale. "Base fertility" means the expected relative fertility of the given soil variety, with limited material and energy inputs. In this context, "base fertility" is similar to what FAO (1996) describes as "low inputs land utilization type".

The actual relative fertility of the land can be expressed by taking land management conditions (fertilization, tillage method, etc.) into account. All things considered, the actual soil evaluation index may exceed the 100-point value of the extensive soil evaluation index based on the "natural" productivity (base fertility) of the soil. With the same method, the effect of any other factor influencing the fertility of soils (e.g. irrigation) could theoretically be incorporated in the evaluation process.

Factors related to environmental quality can be expressed through the soil evaluation system in the following way: the productivity of the genetic soil variety is evaluated by a process similar to that described above, but the soil evaluation indices are expressed as a percentage of the national mean productivity value (and not on a 100-point scale). The calculated index shows the natural (or base) fertility of the investigated land in comparison to the expected base fertility when all Hungarian croplands are considered.

In addition to the relative natural fertility index, environmental quality (or environmental risk) is also indicated by symbols or abbreviations and by numbers highlighting the magnitude of the risk, given in brackats after the natural fertility index. For example: 0.67 (2er, 1ac), where the first number shows that the natural (base) fertility of the soil in question is 67% of the national average, with a medium level of erosion risk (2er) and a slight risk of acidification (1ac).

A complex soil evaluation description can be achieved by aggregating the indices described above:

Wheat 98 [0.67 – 2er, 1ac]

This index highlights the relative fertility of the investigated field if the cultivated plant is wheat, under the given land management, fertilization, etc. conditions. The index also shows the relative production potential of the land under extensive management practice and indicates the forms and levels of ecological risks.

Concluding remarks

To optimize agricultural plant production, economic benefits have to be considered in relation to environmental criteria. The economic return of crop production can be calculated on the basis of the energy input-output ratio. The yield level of cultivated fields, which is the main component in the energy output of cropping systems, varies not only according to the level of inputs (fertilization, irrigation, mechanization, weed and pest control, labour, etc.), but also according to the soil. Different soil types convert, store and release material and energy in different ways. The type of soil also determines the type of environmental risk to which the cultivated field may be exposed. Fertility and other soil quality parameters can be described through a combination of certain soil attributes. The soil productivity assessment method outlined above is based on the genetic soil classification and takes the relevant soil characteristics into account. The result is a productivity rating system which could help to integrate various land quality parameters, such as productivity and ecological sensitivity, in a complex land evaluation framework, thus providing a tool for the planning of best practices for agricultural land use.

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EVALUATION OF BRAY-1 METHOD FOR ESTIMATING PLANT P AVAILABILITY IN THE TROPICAL SOILS OF NIGERIA

A.Y. ADEPOJU and F. A. AFOLABI

NATIONAL CEREALS RESEARCH INSTITUTE, BADEGGI, NIGERIA

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Surface soil materials from 12 sites were each incubated with a known quantity of P for 6 weeks, after which the availability was estimated using 5 different chemical extractants. The soils were then cropped in the greenhouse to determine actual plant P removal and to correlate the chemical test values with total plant P removal. The Bray-1 and -2 methods extracted as high as 96 to 100% of the added P, while the North Carolina, HCl and Olsen bicarbonate methods gave maximum extractions of between 67 and 77%. Only the Bray-1 and -2 values seemed to show a definite pattern regarding the soil properties, with the Bray-1 providing a better relationship for predicting plant P removal. The percentage of added P extractable by Bray-1 correlated positively with organic matter and clay + silt, while that extractable by plant removal correlated positively with pH and soluble Ca. The recovery of added P by Bray-1 was much greater in the slightly acid rainforest soils than in the forest and savanna soils.

In general, the Bray-1 method seemed to be suitable for estimating P availability to plants in moderately acid to neutral soils, but appeared to overestimate P availability in very acid soils of pH 4.6.

Key words: P availability, chemical extractants, plant P removal, Bray-1 method, soluble Ca, rainforest zone, savanna zone, neutral soils, very acid soil

Introduction

Phosphorus is among the most needed elements for crop production and its availability in soils is generally estimated by the use of chemical extractants. However, the difference in the distribution of various forms of P, together with the diversity of P forms and their relative proportions in soils, as dictated by parent materials, weathering intensities and even long-term management practices, have necessitated the use of different P extractants for different soil areas (Chang and Jackson, 1958).

The most commonly used P extractants for soils in the tropics are Bray-1, the North Carolina and the Olsen bicarbonate. They are all effective in extracting both Ca-P and Al-P, and the Olsen bicarbonate is in addition sensitive to Fe-P (Sanchez, 1976). In general, Nigerian soils are acid to neutral in reaction and are therefore likely to contain mainly Al-P and Fe-P (Uzu et al., 1975).

The common practice has been the use of the Bray-1 method in estimating available P in Nigerian soils, but there have been frequent reports of viable agricultural soils giving relatively low Bray-1 values. Uzu et al. (1975) reported that 17 out of 21 soils collected across the country and tested for Bray-1 available P exhibited, on average, values of less than 3 ppm, with 0 ppm for 4 of the soils. Obigbesan and Mengel (1981) also recorded values of 3 and 2 ppm, respectively, as the Bray-1 P for Oban and Umunyi agricultural soils in the rainforest zone of Nigeria. In yet another study, Enwezor (1977) found some agricultural soils in South-Eastern Nigeria to have values as low as 3.6 ppm. All these values fall within the very low to low range of the original calibration developed by Bray and Kurtz (1945).

Instances of little or no response to P applied to soils have been observed, and where such soils are of relatively high organic matter content, the lack of response was simply attributed to the mineralization of organic P (Uzu et al., 1975; Adepetu and Corey, 1977; Osiname, 1979). This calls to question the suitability of the Bray-1 method for Nigerian soils.

The objective of this study was therefore to assess the suitability of the Bray-1 method, in relation to other extractants, in predicting soil-P availability to plants.

Materials and methods

Bulk samples of surface soil materials (0–15 cm depth) were collected from 12 different locations in Nigeria. The samples were air-dried and passed through a 2 mm sieve, after which each sample was mixed and a sub-sample was taken for laboratory analyses.

The soil samples were analysed for various constituents and characteristics using common laboratory procedures. The pH was measured in a 1:2 soil to water dilution. Mechanical analysis was made by the hydrometer method (Day, 1965). Organic carbon was determined by the dichromate oxidation method of Walkley and Black (1934). Exchangeable cations were extracted with neutral NH_4OAc, and the contents of K and Na in the extracts were determined with a flame photometer, while an atomic absorption spectrophotometer was used for Mg and Ca determinations. The calcium carbonate equivalent was determined by the acid neutralization method (U.S. Salinity Laboratory Staff, 1954). Iron and aluminium oxides were determined by the method of Jackson (1974).

The available P in the soils was analysed using the methods Bray-1 (0.03 N NH_4F + 0.025 N HCl), Bray-2 (0.03 N NH_4F + 0.1N HCl), North Carolina (0.025 N H_2SO_4 + 0.05N HCl), HCl (0.3 N HCl) and Olsen bicarbonate (0.5 M $NaHCO_3$ at pH 8.5). In each case, the phosphorus in the extracts was determined by the ascorbic acid method (Murphy and Riley, 1962).

Five-kilogramme samples of the air-dried soils were weighed into plastic pots. Two pots were prepared for each of the 12 soils. Phosphorus, as potassium dihydrogen phosphate (KH_2PO_4) was applied in solution to each soil at P_0 (control treatment) and P_1, equivalent to 0 and 30 kg P/ha, respectively. The water content was adjusted to 60% saturation and the samples were incubated at room temperature. The soil moisture was kept constant by weighing and adding more water to replace the quantity lost through evaporation. After the 6-week incubation period, small samples were taken from the pots, air-dried and analysed for available P, using the various extractants. The quantity of added P removed by an extractant was taken as the difference between the P extracted from the control soil and that extracted from the treated soil.

The pots were then moved to the greenhouse, where N and K were applied at rates equivalent to 75 kg N/ha and 25 kg K/ha, to ensure that the nutrient elements were not limited during cropping. Maize (*Zea mays* L.) was planted and after emergence, the plants were thinned to 4 plants/pot. The soils were cropped twice. During each cropping, plant tops were harvested 5 weeks after planting, after which the roots were carefully removed and soil particles washed off. All the harvested materials were air-dried and then oven-dried at 60°C to constant weights.

The dried plant materials were milled and digested by the perchloric acid method (wet oxidation) for total P determination, using the vanadate molybdate yellow method. The quantity of added P removed by the plants was taken as the difference between the total plant P uptake from the control and treated pots of the particular soil type, expressed as a percentage of added P. The data for the plant P uptake from the various soil types were correlated with the P removed by the extractants.

The residual P from the added P was calculated as the difference between the available P in the control and treated soils after the croppings. A balance sheet was then prepared to relate the unaccounted for P to the characteristics of the soils.

Results and discussion

Data on the properties of the soils used in the study are presented in Table 1. The pH values show that the soils were strongly acidic to about neutral, and the values for the clay + silt and the organic matter (O.M.) contents indicate that the soils of the rainforest were relatively fine textured with a high O.M. content, as compared with the forest and derived savanna soils.

The proportions of added P extracted from the soils after the incubation period ranged from 53.3 to 100% for Bray-1, 33.3 to 95.8% for Bray-2, 10.0 to 76.7% for North Carolina, 0.0 to 75.0% for HCl and 0.0 to 66.7% for the Olsen bicarbonate (Table 2). Thus, Bray-1 extracted the highest proportions while Olsen bicarbonate extracted the least proportions of the P added to the soils. Also, only Bray-1 and Bray-2 gave values that seemed to show a definite pattern, regarding the properties of the soils. However, because the Bray-1 values appear to be more effective in predicting P removal, this was selected for more detailed discussion. Throughout the remainder of the paper, Bray-1 extractable P refers to the percentage of added P extracted by Bray-1, unless otherwise stated.

Table 1
Some physical and chemical properties of the soils used

No.	Soil	UC	Vegetation	pH	O.M.	SCa	SMg	$CaCO_3$	Fe+Al	C+S	P
1	Iwo	Alfisol	Derived Savanna	6.8	1.82	1.28	1.51	3.0	1.56	10.0	4.7
2	Ibadan	Alfisol	Forest	6.1	1.89	1.26	1.15	2.50	1.71	8.1	3.3
3	Apomu	Entisol	Forest	6.2	2.06	0.88	1.06	3.20	2.56	5.5	3.6
4	Gambari	Alfisol	Forest	6.3	2.06	0.71	1.71	3.10	2.69	9.5	3.8
5	Jago	Alfisol	Derived Savanna	6.5	1.93	1. 67	0.79	1.80	1.03	12.7	4.9
6	Iregun	Alfisol	Forest	6.4	2.20	0.71	1.45	2.40	1.70	13.6	4.5
7	Akure	Alfisol	Rain-forest	6.9	3.61	5.92	3.30	3.65	6.22	41.4	6.0
8	Amakama	Oxisol	Rain-forest	4.6	4.40	0.56	0.93	1.90	4.88	20.5	6.3
9	Amangwo	Oxisol	Rain-forest	4.6	3.70	0.64	0.72	1.85	3.41	19.3	8.3
10	Ajasse	Oxisol	Savanna	5.9	2.37	1.77	2.22	2.00	1.00	10.5	8.9
11	Mokwa	Entisol	Savanna	6.7	2.41	3.00	0.46	1.90	1.36	9.8	8.5
12	Kulfo	Entisol	Rain-forest	6.2	4.51	3.00	3.21	2.80	2.04	17.0	8.2

UC: USDA Classification; O.M.: Organic matter (%); SCa: Soluble Ca (me/100 g); SMg: Soluble Mg (me/100 g); Fe + Al: Fe + Al oxides (%); C+ S: Clay + silt (%); P: Bray-1 P (mg/kg)

Table 2
Percentage of added P removed by the various extractants and by plant uptake

No.	Bray-1	Bray-2	NC	OB	HCl	1st h.	2nd h.	Total u.
1	61.5	41.7	40.0	16.7	50.0	19.2	6.4	25.6
2	63.0	44.7	36.7	20.3	46.7	20.6	7.2	27.8
3	54.2	56.3	36.7	18.5	16.7	13.7	3.2	16.9
4	53.3	33.3	21.7	0.0	8.3	15.3	2.8	18.1
5	66.7	37.5	31.7	16.7	37.5	23.3	11.4	34.7
6	75.0	33.3	10.0	88.5	0.0	24.1	11.0	35.1
7	100.0	75.0	40.0	19.3	33.3	33.2	10.4	43.6
8	95.0	82.0	30.0	20.0	75.0	11.6	1.3	12.9
9	97.5	95.8	46.7	33.3	75.0	9.2	1.5	10.7
10	95.5	91.7	50.0	50.0	58.3	31.8	13.0	44.0
11	95.8	79.2	76.7	66.7	54.2	27.9	10.3	38.2
12	100.0	95.8	60.0	60.0	75.0	31.5	11.2	42.7

NC: North Carolina; OB: Olsen bicarbonate (%); 1st h.: 1st harvest; 2nd h.: 2nd harvest; Total u.: total uptake

Linear correlation coefficients for relationships between various soil properties, and between the soil properties and both Bray-1 extractable P and total P removal by crops, are presented in Table 3. The Bray-1 extractable P correlated positively with the clay + silt and the O.M. contents, but gave no significant correlations with other soil parameters, including soluble Ca. This seems to suggest that the only significant factors for the adsorption of applied P in soils are the texture and O.M. content. In general, the moderately acid rainforest soils showed greater recovery of added P than the forest and savanna soils, with relatively less fine-textured particles and low O.M. content.

Judging by the relatively short period of time for which this study was carried out, the fertilizer-reaction product resulting from the added P could not have been transformed beyond the least stable Ca phosphate, so the solubility should also be a function of Ca activity (Aslyng, 1954; Adepoju et al., 1986). The low correlation obtained for the relationship between Bray-1 and soluble Ca in this study was therefore investigated further by plotting the graph of the soil parameters (Fig. 1). The plotted points suggest that the soils could be separated into two groups to look at the correlation in greater detail.

Consequently, soils 7, 8, 9, 10, 11 and 12 were considered as group 1 and soils 1, 2, 3, 4, 5 and 6 as group 2. The group 1 soils, which were mostly rainforest soils with relatively high O.M. content (2.41–4.4%), gave almost 100% desorption of added P, regardless of the level of soluble Ca contained in the soils. This observation is in agreement with the reports of earlier workers (Olsen and Watanabe, 1970; Gerke, 1992; Ronvaz et al., 1993; Akonde et al., 1999) that manure-treated plots gave more soluble P than other plots, including those treated with inorganic P fertilizers. The higher soluble P concentration with manure was attributed to the fact that manure contains a mixture of organic

P forms, including inositol penta- and hexaphosphates (IPP and IHP), while Anderson et al. (1974) showed that IHP adsorbs on the same sites in acid soils as $H_2PO_4^-$ ions. The competition with $H_2PO_4^-$ ions on the adsorption sites would no doubt lead to a higher concentration of $H_2PO_4^-$ ions in solution. Also, the finer the soil texture the greater the number of sites for anion exchange activity.

Table 3
Linear correlation coefficients for relationships among soil characteristics including percentage of added P extracted by Bray-1 and P removed by cropping

Variable	O. M.	SCa	SMg	$CaCO_3$	Fe+Al	C+S	PB-1	PC
pH	–0.535	0.552	0.331	0.523	–0.030	-0.030	0.309	0.634+
O.M		0.273	0.388	0.057	0.624*	0.626*	0.768**	–0.032
SCa			0.658*	0.418	0.386	0.724*	0.513	0.739**
SMg				0.619*	0.322	0.552	0.338	0.584
$CaCO_3$					0.393	0.299	0.306	0.083
Fe+Al						0.832**	0.362	–0.243
C+S							0.605*	0.237
PB-1								0.393

O.M.: Organic matter; SCa: Soluble Ca; SMg: Soluble Mg; Fe +Al: Fe+Al oxides; C+S: Clay+Silt; PB-1: % added P extracted by Bray-1; PC: % added P removed by cropping; Symbols * and ** indicate statistically significant correlations at the 0.05 and 0.01 probability levels, respectively. All other coefficients are not significant at the 0.05 probability level.

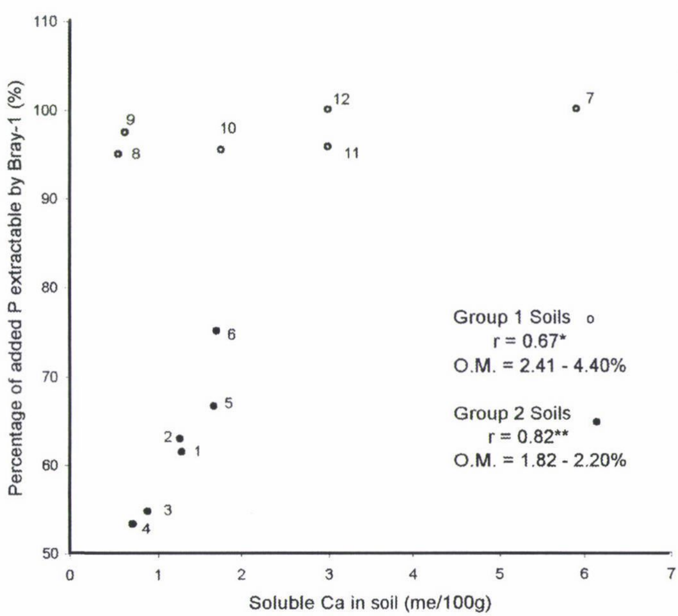

Fig. 1. Relationship between percentage of added P by Bray-1 and soluble Ca content of soil. Numbers by data points indicate soil numbers presented in Table 1.

The group 2 soils contained relatively low O.M. (1.82–2.20%) and the significant correlation for the group (r = 0.823) seems to suggest that the adsorption of applied P is also dependent on the soluble Ca level, particularly in soils with low O.M. content.

The proportions of added P removed by plant uptake during the croppings were much greater in the first harvest than in the second harvest (Table 2), obviously reflecting the higher soil P level during the first cropping. The two harvests, however, showed similar trends of plant P uptake. Except for the strongly acidic soils 8 and 9, P removal by the crop generally reflected the level of P availability, as estimated by the Bray-1 method. Table 4 shows that when all soils were considered, the relationship between the percentage of added P extracted by Bray-1 and that removed by plant uptake produced correlation coefficients of 0.414, 0.291 and 0.375 for the first and second harvests, and the total harvest, respectively. The respective values, however, improved to the highly significant values of 0.968, 0.794 and 0.935 when the very acid soils 8 and 9 (pH 4.6) were not considered.

Although the Bray-1 extractable P values for soils 8 and 9 indicate that large proportions of added P would be available for plant uptake, the actual P removals by crops in the soils were very low, when compared with soils 7, 10, 11 and 12, which had similar Bray-1 extractable P levels. The data in Table 3 show that the only significant factors in the removal by crops of the added P were the pH and the soluble Ca. For instance, soils 8 and 9, which had the lowest values for soil pH and soluble Ca, gave the least P removal. At the pH 4.6 value of soils 8 and 9, aluminium and manganese are highly soluble and the soil solutions might possibly contain Al^{3+} and Mn^{2+} ions at levels toxic to plants. Studies reported by Goss and Carvalho (1992) and Fageria et al. (1990) indicate that toxic levels of Al and Mn generally impede the uptake and translocation of many essential nutrients, including Ca and P, to plant tops. Therefore, the Bray-1 method could not adequately predict soil-P availability to plants, since toxic levels of Al and Mn in soils 8 and 9 would prevent P uptake. This also seems to explain the relatively high residual P found in the two soils after the croppings (Table 5).

Table 4

Linear correlation coefficient between percentage of added P removed by extractants and by plant uptake

No.	pH range	Cropping	Bray-1	Bray-2	NC	O	HCl
12	4.6–6.9	1stharvest	0.414	0.198	0.383	0.488	0.000
10	5.9–6.9		0.968**	0.754*	0.493	0.641	0.569
12	4.6–6.9	2ndharvest	0.291	0.035	0.264	0.422	–0.063
10	5.9–6.9		0.794*	0.511	0.316	0.548	0.472
12	4.6–6.9	Total	0.375	0.143	0.264	0.422	–0.025
10	5.9–6.9		0.935**	0.691*	0.445	0.627	0.551

NC: North Carolina; O: Olsen; *,**: Significant at the 0.05 and 0.01 probability levels, respectively.

Table 5
Balance sheet for the added P

Soil No.	Total plant P uptake	Residual soil P (%)	P unaccounted for
1	25.6	0.0	74.4
2	27.8	0.8	71.4
3	16.9	1.6	81.5
4	18.1	1.6	80.5
5	34.7	0.8	64.5
6	35.1	0.0	64.9
7	43.6	2.5	53.9
8	12.9	9.2	77.9
9	10.7	5.8	83.5
10	44.8	1.7	53.5
11	38.2	0.0	61.8
12	42.7	4.2	53.1

The negative correlation between unaccounted for P and soluble Ca in Table 5 suggests that soils with high Ca contents are likely to release more P for plant uptake and have less P for possible transformation into insoluble P compounds. A number of researchers (Larsen and Widdowson, 1971; Adepoju et al., 1986) have reported the transformation of less stable immediate reaction P products with time into less soluble reaction P products.

The results, in general, show that while plant P availability, as estimated by Bray-1, seemed to be a function of soil texture and O.M., the actual plant P uptake appeared to be dependent on pH and soluble Ca.

On the whole, the study showed that the Bray-1 extraction method is suitable for the estimation of available P in moderately acid to neutral soils only.

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EFFECT OF DIFFERENT SOWING METHODS ON YIELD AND BULB CHARACTERISTICS IN ONION (*ALLIUM CEPA* L.)

S. MASSIHA, A. MOTALLEBI and F. SHEKARI

DEPARTMENT OF HORTICULTURAL SCIENCE, FACULTY OF AGRICULTURE, TABRIZ UNIVERSITY, TABRIZ, IRAN

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Direct sowing (DS), row direct sowing (RDS), and the transplanting method (TM) were investigated for two onion cultivars, Azar-Shahr (red skin onion) and Horand (yellow-brown skin onion) during 1999, at Khalat-Pooshan Research Station, Tabriz University. Analysis of variance for the measured traits indicated that except for the percentage of class II onions, other traits were significantly influenced by the sowing methods. TM had higher values of total yield, marketable yield, percentage of class I and III, bulb weight, homogeneity (HOM) of bulb weight, bulb diameter, HOM of bulb diameter, bulb length, HOM of bulb length, bulb diameter at the neck and base, number of centres, and percentage of bulbs containing multiple centres than the other methods. The cultivars differed significantly only for bulb weight, HOM of bulb weight, and postharvest longevity characteristics. The data suggest that TM could be an effective method in onion production areas without causing a change in soil texture. RDS was also a better method than DS in terms of marketable yield, and homogeneity of bulb weight.

Key words: *Allium cepa* L., onion, direct sowing (DS), row direct sowing (RDS), transplanting method (TM), bulb characteristics, marketable yield

Introduction

The transplanting method (TM) is more common than direct sowing (DS) in onion production and research in developed countries (Dobrzanski, 1973; Orkwor et al., 1981; Comrie, 1986. Ahmed et al., 1990; Brewster, 1990; Muro et al., 1991. Dobrzanski et al., 1995; Singh and Tiwari, 1995; Gupta et al., 1996; Sinha et al., 1996; Daymond and Wheeler, 1997). TM has a beneficial effect on onion production in five important ways: it provides better establishment of seedlings, earliness, homogeneous bulbs and higher yield than DS and also prevents a change in soil structure.

In trials with the onion cvs. Yellow Bermuda and Granex the highest yields resulted from TM and the lowest from DS (Ramtohul and Splittstoesser, 1979). Pessala (1990) carried out experiments at 2 sites in Southern Finland in 1987 and 1988 and concluded that DS gives lower class I (>5 cm in bulb diameter) yield (2–3 t/ha) than TM (35–40 t/ha). Huerres-Perez (1978) reported that onions transplanted in early December gave the highest total yield in both years (1976–1977) in Cuba. Warid and Loaiza (1993) indicated that DS gave a lower overall average yield than TM (36.2 t/ha for DS vs. 44.1 t/ha for TM) in Mexico. Torres et al. (1986) showed that with TM the yield was 20.37–35.86 t/ha and with DS it was 10.35–10.38 t/ha in the State of Santa Catarina, Brazil. Khokhar et al. (1990) obtained data on bulb maturity, bulb weight and yield either directly in the field or in the nursery followed by transplanting at 3 dates for each method. The differences between methods and dates were highly significant. TM gave better results for bulb yield (5.2–31.4 t/ha) and bulb weight (80.5–441.4 g) than DS (6.4–26.8 t/ha and 20.8–177.8 g, respectively). Ramtohul and Splittstoesser (1979) showed that a higher percentage of large bulbs (>5 cm in bulb diameter) was obtained from TM than DS. Vik (1974) indicated that the crop matured 13 days earlier than in DS.

Bartos and Holik (1986) raised seedlings of cv. Forta F-1 in plastic pots and transplanted them after 7 weeks. This method produced 56.17% higher yield and 63.52% higher sale price at a poor growing site than the DS (control) crop. In a favourable locality the yield and price differences between TM and DS were lower. These authors suggested that until planting machines become available TM should only be used in marginal conditions.

On the other hand, Gaafer et al. (1979) reported that DS produces a higher total yield, but lower yields of export quality onions than TM in Egypt. DS also produced an earlier crop to TM. In addition, it increased the total soluble solids and acidity of the bulbs in Saudi Arabia (Abdalla et al., 1980).

In onion production areas of Iran, the farmers are not aware of the advantages of TM. They sow onion seeds as DS and then cover them with sand in order to facilitate germination, emergence of seeds and seedling establishment. This method is one of the most important causes of changes in soil texture in onion production areas. In addition, the bulbs have larger variation in diameter and weight. Since there has been no report on row direct sowing (RDS) the present study was aimed at comparing the three sowing methods of DS, RDS and TM for yield and bulb characteristics.

Materials and methods

The seeds of 2 cultivars, Azar-Shahr (red skin onion) and Horand (yellow-brown skin onion) were sown on 13 February 1998 in the nursery. The seedlings were transplanted when they were 60 days old and had the characteristics shown in Table 1.

The factorial experiment was conducted using a randomized complete block design with four replications. The factors included three sowing methods (DS, RDS and TM) and two cultivars (Azar-Shahr and Horand). Each plot was 1.2 m in width and 6 m in length (for RDS and TM, each plot contained 6 rows).

On 13 April 1999, the sets of the two cultivars were sown at a rate of 25 kg/ha (with a final density of 5 × 5 cm or 400 plants/m^2) in DS and 5 kg/ha (with a final density of 20 × 7 cm or 72 plants/m^2) in RDS, and the seeds were then covered with sand at 62 kg/plot in all the DS plots and at 26.5 kg/plot on the sowing rows only in RDS The seedlings of the two cultivars were also transplanted at 20 × 10 cm or 25 plants/m^2 at this date for TM.

Table 1
Seedling characteristics (after 60 days)

Characteristics	Length of seedling (cm)	Bulb diameter (mm)	Diameter at neck (mm)	Length of roots (mm)	Number of roots	Number of leaves
Mean	18	4.24	2.615	47.37	9.70	2–3
Standard error	1.7	0.294	0.13	3.11	1.06	–

In September 1999, the bulbs of each plot were harvested separately and weighed after one week. Forty bulbs were chosen randomly from each plot and were measured for diameter and length of bulbs, bulb diameter at the neck and base, bulb weight, bulb diameter to length ratio, number of centres, and percentage of bulbs containing multiple centres (PMC). The homogeneity of the traits was also measured by the following equations:

Homogeneity (HOM) = 100 – CV%, CV% (Coefficient of Variation) = S/X × 100,

where S = Standard deviation of the trait, and X = mean for trait.

The classes of bulbs and marketable yield were defined as follows:

Class III: bulb diameter < 5 cm

Class II: 5 < bulb diameter < 7 cm

Class I: bulb diameter > 7 cm

Marketable yield (MY) = Percentage of bulbs with diameter >5 cm × total yield.

The data for the percentage of class I, II and III bulbs, the HOM of all traits, the diameter to length ratio, the number of centres, and the percentage of bulbs containing multiple centres (PMC) were transformed according to procedures described by Steel and Torrie (1991) to improve normality. After analysis of variance the means of treatments and factors were compared using Duncan's multiple range test.

Results and discussion

Total and marketable yields

The total and marketable yields were significantly influenced by the sowing methods, but the two cultivars did not differ in these respects (Table 2). The sowing method × cultivar interactions were not significant (Table 2). The average total yield increased from 41.558 and 45.517 t/ha in RDS and DS, respectively, to 70.56 t/ha in TM. This agrees with the reports of other researchers (Huerres-Perez, 1978; Ramtohul and Splittstoesser, 1979; Torres et al., 1986; Pessala, 1990; Khokhar et al., 1990; Warid and Loaiza, 1993). Also, the average marketable yield increased from 14.9 t/ha in DS to 66.11 t/ha in TM (Table 2). Ramtohul and Splittstoesser (1979), Kudo (1996) and Gaafer et al. (1979) observed that TM resulted in higher marketable yield than DS. Furthermore, TM had a significantly higher percentage of class I and II bulbs (56.27 and 36.27, respectively) than the other methods (Table 2) (Gaafer et al., 1979; Ramtohul and Splittstoesser, 1979; Kudo, 1996).

Bulb characteristics

Analysis of variance for marketable bulb characteristics and homogeneity (HOM) are shown in Table 3. Except for bulb weight and HOM of bulb weight, there were no significant differences between the two cultivars for other bulb characteristics and HOM (Table 3). Bulb weight was greater in Azar-Shahr (87.68 g) than in Horand (73.72 g). The sowing methods significantly influenced bulb diameter, HOM of bulb diameter, bulb length and HOM of bulb length, but the shape of the bulb, or diameter to length ratio (d/l), was not significantly affected by the sowing methods (Table 3). Sowing method × cultivar interactions were significant for bulb length and bulb weight but no significant differences were observed in the other interactions (Table 3). Khokhar et al. (1990) indicated that TM gave better results for bulb weight than DS. TM produced larger, more homogeneous bulbs than the other methods for both cultivars (Table 3).

Table 2

Effects of sowing methods and cultivars on total and marketable yield and percentage of class I, II and III bulbs

Factors	Trait df	Total yield (t/ha)	Mark⁺. yield (t/ha)	Class I (%)	Class II (%)	Class III (%)
Sowing methods						
DS		45.51 b⁺	14.90 b	2.287 b	31.02 a	66.86 b
RDS		41.55 b	22.47 b	4.175 b	49.43 a	46.38 b
TM		70.56 a	66.11 a	56.27 a	36.27 a	7.438 a
Cultivars						
Azar-Shahr		52.71 a	31.62 a	26.32 a	33.97 a	39.7 a
Horand		52.38 a	30.90 a	15.50 a	43.85 a	40.6 a
Source of variation						
Block	3	67.78 ns	130.3 ns	288.6 ns	142.34 ns	0.051 ns
Sowing methods (SM)	2	1978.6***	6111.9***	7204***	7170***	1.124***
Cultivars (C)	1	0.6530 ns	18.69 ns	4.8600 ns	575.26 ns	0.003 ns
SM × C	2	9.8730 ns	111.9 ns	132.01 ns	418.865 ns	0.018 ns
Error	15	117.008	137.38	114.53	298.083	0.022

⁺: Mark.: Marketable; *** significant at p< 0.001; ns: non-significant; ⁺: Means within each column were compared by Duncan's New Multiple Range Test, p<0.01; HOM = homogeneity.

Table 3

Effects of sowing methods and cultivars on bulb weight, HOM of bulb weight, bulb diameter, HOM of bulb diameter, bulb length, HOM of bulb length, bulb shape and HOM of bulb shape

Factors	Trait df	BW (g)	HBW (%)	BD (mm)	HBD (%)	BL (mm)	HBL (%)	BS	HBS (%)
Sowing methods									
DS		39.719b⁺	74.5 c	45.980 b	79.05 b	35.844 b	83.5 b	1.281 a	82.33 a
RDS		54.303 b	82.7 b	51.519 b	80.30 b	38.335 b	83.01 b	1.357 a	83.48 b
TM		148.084a	85.2 a	69.512 a	85.21 a	53.404 a	86.5 a	1.339 a	88.22 a
Cultivars									
Azar-Shahr		87.684a	81.66 a	56.850 a	80.95 a	43.128 a	83. 9 a	1.327 a	86.95 a
Horand		73.720 b	78.54 b	54.491 a	81.96 a	41.927 a	84.7 a	1.324 a	85.00 a
Source of variation									
Block	3	467.97 ns	2.056 ns	59.3 ns	20.717 ns	15.1 ns	6.916 ns	0.007 ns	0.173 ns
Sowing methods	2	27667.2***	219.2***	1210.9***	82..95***	722.1***	29.078 *	0.013 ns	0.622 ns
Cultivars	1	1170.086*	32.67***	3.33 ns	6.378 ns	8.60 ns	3.490 ns	0.001 ns	0.530 ns
SM × C	2	137.079ns	1.29 ns	50.93 ns	20.894 ns	72.31***	10.47 ns	0.021 ns	0.384 ns
Error	15	228.856	1.722	20.786	7.302	5.57	8.108	0.006	0.692

BW: Bulb weight;HBW: HOM of bulb weight; BD: Bulb diameter: HBD: HOM of bulb diameter BL: Bulb length; HBL: HOM of bulb length; BS: Bulb shape (D/L); HBS: HOM of bulb shape; *, *** significant at p <0.05 or p< 0.001, respectively; ns non-significant; +: Means within each column were compared by Duncan's New Multiple Range Test, p<0.01; HOM = homogeneity.

Bulb characteristics related to postharvest longevity

Bulb characteristics related to postharvest longevity, such as the diameter at the neck and base of the bulbs, the number of centres and the percentage of bulbs containing multiple centres (PMC), were significantly influenced by sowing methods and cultivars (Table 4). TM may thus lead to lower postharvest longevity than the other methods, because it produces larger bulbs with a greater diameter at the neck and base, a larger number of centres and a greater percentage of bulbs containing multiple centres (PMC) (Table 4). It seems that the Azar-Shahr cultivar had better postharvest longevity than Horand (Table 4).

The data suggest that TM could be an effective method of producing onions without a change in soil texture. RDS is also better than DS because it has little influence on the soil texture and gives higher marketable yield and better homogeneity of bulbs.

Table 4

Effects of sowing methods and cultivars on the diameter of the neck and base of the bulbs, the number of centres and the percentage of bulbs containing multiple centres (PMC)

Factors / Trait	Diameter at neck	Diameter at base	Number of centres	PMC
Sowing methods				
DS	11.666 b [+]	14.039 c	1.060 c	5.354 c
RDS	13.937 b	15.647 b	1.195 b	17.614 b
TM	19.286 a	18.871 a	1.689 a	57.697 a
Cultivars				
Azar-Shahr	13.667 b	15.719 b	1.222 b	21.536 b
Horand	16.259 a	16.652 a	1.407 a	32.240 a
Source of variation				
Block	1.828 ns	0.225 ns	0.007 ns	122.837 ns
Sowing methods (SM)	122.44***	48.445***	0.876***	5995.6***
Cultivars (C)	40.300***	5.227*	0.204***	687.51***
SM × C	5.286 ns	1.185 ns	0.024 ns	140.434 ns
Error	1.970	0.681	0.006	50.834

ns: non-significant; * and ***: significant at $p<0.05$ or $p< 0.001$, respectively; + : Mean separation within each column by Duncan's Multiple Range Test, $p<0.05$ or $p<0.001$.

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ASSOCIATION OF CHARACTERS AND PATH COEFFICIENT ANALYSIS OF SEED YIELD AND YIELD COMPONENTS IN ONION (*ALLIUM CEPA* L.)

S. AKLILU[1], L. DESSALEGNE[1] and L.CURRAH[2]

[1]ETHIOPIAN AGRICULTURAL ORGANIZATION, MELKASSA AGRICULTURAL RESEARCH CENTER, NAZARETH, ETHIOPIA
[2]HORTICULTURE RESEARCH INTERNATIONAL, WELLESBOURNE, STRATFORD-UPON-AVON, UK

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Fifteen onion genotypes (one standard check and 14 exotic cultivars) were evaluated in RCBD with 3 replications at Melkassa Research Center during the 1999/2000 growing season (Aug.–Feb.). The objective of the study was to understand the association of characters with seed yield.

The genotypic correlation coefficients were greater in magnitude than the phenotypic ones. Seed yield/plant had a high, significant correlation with number of flower stalks/plant, number of seeds and flowers/umbel and umbel size. Bolting and flowering period had a significant negative correlation with seed yield/plant. From the path analysis results, the number of flower stalks/plant, bolting period, thousand seed weight, flower stalk diameter and umbel size had a high direct positive effect on seed yield/plant. Since the direct and indirect effects through these components on seed yield are high and positive, selection should concentrate on these traits for high seed yield in onion cultivars. Since these components were found to affect seed yield they could be used for developing varieties for the growing onion industry in the country.

Key words: onion, path coefficient

Introduction

Onion is a recently introduced crop in the agricultural community of Ethiopia. Its introduction dates back to the early 1970s when it was brought in by foreigners. Though shallots are a traditional crop in Ethiopia, onions have become more widely grown in recent years. In the last few years the demand for onion has increased significantly due to its high bulb yield, seed and flower production potential. Most tropical countries import much of their onion seed from temperate countries where the winter season provides the chilling requirements which induce optimal flowering. There is also great difficulty in obtaining a large number of easy flowering cultivars. However, in Ethiopia onion seed is produced from the easy flowering cultivar Adama Red, without any pretreatment during the cool season (Oct.-Jan.) of the year. At present many free flowering cultivars are available at Melkassa Research Center through the International Collaborative Onion Variety Trials carried out with Horticulture Research International (HRI), Wellesbourne, UK. Information regarding the flower development and seed yield is meagre and no systematic study has yet been done on the interaction of complex characters that could significantly affect the seed yield of onion cultivars in Ethiopia.

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A knowledge of the association of certain plant characters with seed yield and among themselves is of paramount importance for improving complex characters like yield. The evaluation of genetic and phenotypic associations of characters may help in determining the extent of improvement to be made in some quantitative traits.

It is important to determine the relationships between various traits which are useful to identify potential characters for selection programmes. Hence, understanding the association of flower components with seed yield will be an important step towards the development of an efficient seed production programme. This work is, therefore, proposed with the objective of studying the association of characters with the seed yield and understanding the contribution of each component to seed yield in onions.

Materials and methods

The experiment was conducted under irrigated conditions at Melkassa Agricultural Research Center, which is located at 8°24 N latitude and 39°12 E longitude at an altitude of about 1550 m.a.s.l. in the Middle Rift valley. It is characterized by low and erratic rainfall with an average of 770 mm per annum with the peak in July and August. The soil is mainly sandy loam with a pH value of 6.5–7.6. The maximum and minimum temperature of the center is 28°C during the warm and 14°C during the cold period, respectively, and the mean temperature over 25 years (1973–1999) is 19°C.

Medium bulbs of fifteen cultivars with uniform diameter (5–6 cm), typical size and colour, free from insect and mechanical injuries were selected in July 1999 and stored under ambient conditions. The bulbs were planted at the end of August 1999. The spacing used was 50 cm between water furrows, 30 cm between rows on the bed and 20 cm between plants in the rows. The plot size was 4 m × 3.2 m. The experiment was laid out in a random complete block design with three replications. Four rows were planted for each variety in each replication. Five plants from the middle two rows were randomly selected from each plot and tagged to record data on flower stalk height and diameter, umbel size, number of flowers and seeds/umbel, and seed yield/plant. Since there was rainfall at the initial stage of crop development the recommended chemical mancozeb was applied 4 times at a rate of 3 kg/ha in 600 litres of water. Fertilizer (DAP 200 kg/ha) was applied before planting and urea (100 kg/ha) was side-dressed one and half months after planting. The seed was harvested when 5 to 10% of the umbels showed open seeds.

Associations between yield and its components were determined by the application of correlation and path analysis as per the method of Dewey and Lu (1959). Analysis of variance was carried out for each variable and covariance analysis was conducted for each pair of variables, using the following equations:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

where σ^2_p = phenotypic variance, σ^2_g = genotypic variance and σ^2_e = environmental variance (error mean square);

$$\sigma^2_g = (\mathrm{msv} - \mathrm{mse})/r$$

where msv = mean square of the varieties, mse = error mean square and r = number of replications;

$$r_g = g_{covxy} / \sqrt{\sigma^2_{gx}}\,\sqrt{\sigma^2_{gy}}$$

where r_g = genotypic correlation, g_{covxy} = genotypic covariance of x and y, σ^2_{gx} = variance of character x and σ^2_{gy} = variance of character y.

Path coefficient analysis was determined by the formula:

$$r_{ij} = P_{ij} + \Sigma r_{ik} P_{kj}$$

where r_{ij} = mutual association between the independent character (i) and dependent character (j) as measured by the genotypic correlation coeffecient, P_{ij} = direct effects of the independent character (i) on the dependent character (j) as measured by the genotypic path coefficients, and $\Sigma r_{ik} P_{kj}$ = summation of components of indirect effects of a given independent character (i) on the given dependent character (j) via all other characters (k).

Results and discussion

The phenotypic and genotypic correlation coefficients for different pairs of characters and yield are presented in Table 1. The phenotypic and genotypic correlation coefficients were close to each other in most instances, probably due to low environmental variance. However, in most cases the genotypic correlations were higher in magnitude than the phenotypic ones, and a number of interesting relationships were observed at genotypic level which showed the inherent associations between various characters. Therefore, in this paper reference will be made only to genotypic correlation to avoid unnecessary repetition.

Table 1

Genotypic (G) and phenotypic (P) correlation coefficients of 10 pairs of characters

		2	3	4	5	6	7	8	9	10
1	G	0.751**	0.158	0.841**	−0.102	0.271	−0.576*	−0.491	−0.715**	−0.512
	P	0.558.*	0.190	0.828**	−0.101	0.207	−0.408	−0.605*	− 0.589*	−0.438
2	G	1	0.217	0.811**	0.089	0.175	−0.821**	−0.431	0.145	−0.561*
	P		0.263	0.604	0.021	0.017	−0.427	−0.207	0.108	−0.555*
3	G		1	0.968**	0.715**	0.708**	0.201	0.204	0.375	0.281
	P			0.905**	0.545*	0.046	0.158	0.200	0.173	0.341
4	G			1	0.414	0.388	−0.801**	0.281	0.475	0.313
	P				0.308.	0.120	−0.278	0.152	0.408	−0.166
5	G				1	0.719**	0.239	0.249	0.815 **	0.692**
	P					0.040	0.015	0.060	0.058	0.421
6	G					1	0.316	0.907**	0.729**	0.718**
	P						0.182	0.515	0.314	0.542*
7	G						1	0.401	0.141	0.721**
	P							0.074	0.273	0.509
8	G							1	0.459	1.000
	P								0.241	0.769**
9	G								1	0.569*
	P									0.715**
10										1

** and * significant at the 0.01 and 0.05 level, respectively; 1. Bolting period, 2. Flowering period, 3. Flower stalk height, 4. Flower stalk diameter, 5. Number of flowers/umbel, 6. Umbel size, 7. Number of flower stalks/plant; 8. Number of seeds/umbel, 9. 1000 seed weight, 10. Seed yield/plant.

Seed yield showed a positive association with most components. Highly significant positive associations were obtained with umbel size (r=0.718), number of flower stalks per plant (r=0.721), number of flowers/umbel (r=0.632) and number of seeds per umbel (r=1.000). A significant positive association was also observed for thousand seed weight (r=0.569), which indicates that all these characters are major components for onion seed yield. The number of flower stalks was found to be the most important character for onion seed-yielding capacity, in which large umbel size and number of seeds per umbel also substantially contributed to seed yield per plot. On the other hand, seed yield per plant showed significant negative associations with days to bolting (r=–0.512) and 50% flowering period (r=–0.561). This clearly indicates that as bolting and flowering became later, the seed yielding potential of the cultivars was badly affected and flower development was suppressed, as the conditions favoured vegetative development. Thus, early bolting and flowering cultivars gave high seed yields due to high rates of flower development and the production of a large number of flower stalks that could grow vigorously when the temperature was favourable during the early flowering period.

Significant positive associations were observed between bolting and flowering period, flower stalk height and diameter, umbel size and number of flowers/umbel, flowering period and flower stalk diameter, umbel size and flower stalk height, and umbel size and thousand seed weight.

Flower stalk height had a better positive association with 1000 seed weight than seed number per umbel, but it gave a very weak positive association with yield as opposed to other reports (Prats Perez et al., 1996). However, Sandhu and Korla (1976) showed that seed yield per plant was positively correlated with the number of seed stalks per plant and the number of seeds per umbel, which agreed with the present findings. In addition, Mital and Srivastava (1965) also reported that seed yield was related to the number of flower stalks per plant, though increasing the seed stalks beyond an optimum number did not show a proportional increase in seed yields. It was also true at Melkassa that the number of flower stalks per plant showed a positive significant association with yield.

In general, seed yield per plant showed a significant positive association with most of the characters, except bolting and flowering period, which showed a negative association with yield.

Path coefficient analysis was carried out (Table 2) with a view to partitioning the genotypic correlations into their direct and indirect contributions and to assessing the relative importance of each causal factor affecting seed yield. Of the 9 characters which were treated as causal factors the highest positive direct effect was obtained for thousand seed weight (5.011), followed by flower stalk diameter (3.953), bolting period (3.525), umbel size (2.887) and flower stalk/plant (1.169). Selection based on these characters either individually or in combination could be expected to lead to higher seed yield. However, flower stalk height (–2.068), number of flowers/umbel (–3.543), flowering period (–7.082) and number of seed/umbel (–3.117) had a high negative direct effect on yield. Therefore, it would be advisable to lay stress on umbel size, stalk diameter, bolting period, flower stalk/plant and thousand seed weight in selection programmes for high seed yield in onion cultivars.

The indirect effect of bolting period through flower stalk number/plant, flower stalk height and flowering period was high and negative, but the influence through flower stalk diameter, umbel size, number of seeds and flowers per umbel was high and positive. The net effect in the system of these opposing influences was that 4 positive effects were counter-balanced by 5 negative effects, making the overall correlation between bolting period and yield high and negative. Although the indirect effect of flowering period through stalk diameter, bolting period, thousand seed weight, umbel size and number of seed/umbel was positive, the overall correlation was high and negative due to its negative direct effect. The results indicated that considering flowering period in selection programmes was not important for onion seed yield due to its very high negative direct effect and low positive indirect effect through most other components on seed yield.

Flower stalk height had a high positive indirect influence on seed yield through bolting period, flower stalk diameter, thousand seed weight and umbel size. Flower stalk diameter had a negative indirect influence via flower stalk height, flowering period, number of flowers/umbel and flower stalk number per plant. This was counter-balanced by the high indirect positive influence on umbel size, thousand seed weight, number of seeds/umbel and bolting period that caused the net effect of overall correlation between stalk diameter and seed yield to be positive and moderately high, but not significant. Due to the very low overall association of flower stalk diameter with seed yield it was not thought to be important for the seed yield of onion cultivars at Melkassa. However, flower stalk diameter was revealed by path analysis to be the second most important component in seed yield next to thousand seed weight. Under these circumstances efforts should be made to nullify the undesirable indirect effect in order to make use of the direct effect of stalk diameter.

Table 2

Direct (bold) and indirect effects of 9 pairs of characters on seed yield

	BPD	FPD	FSH	FSD	NFPU	US	NFSPP	NSPU	THSWT	GC
BPD	**3.525**	–5.319	–0.461	3.324	0.361	0.782	–0.673	1.530	–3.583	–0.512
FPD	2.647	**–7.082**	–0.633	3.206	–0.315	0.505	–0.960	1.343	0.727	–0.561
FSH	0.557	–1.537	**–2.918**	3.827	–2.533	2.043	0.235	–0.636	1.248	0.281
FSD	2.965	–5.743	–2.824	**3.953**	–1.477	1.120	–0.936	0.876	2.38	0.313
NFPU	–0.360	–0.630	–2.086	1.648	**–3.543**	2.076	0.279	–0.776	4.084	0.692
US	0.955	–1.239	–2.066	1.534	–2.547	**2.887**	0.369	–2.827	3.653	0.718
NFSPP	–2.030	5.814	–0.587	–3.166	–0.847	0.912	**1.168**	–1.250	0.707	0.721
NSPU	–1.731	3.052	–0.595	–1.111	–0.882	2.619	0.468	**–3.117**	2.30	1.000
THSWT	–2.52	–1.027	–0.724	1.878	–2.887	2.105	0.165	–1.431	**5.011**	0.569

BPD: Bolting period, FPD: Flowering period, FSH: Flower stalk height, FSD: Flower stalk diameter, NFPU: Number of flowers/umbel, US: umbel size, NFSPP: Number of flower stalks/plant, NSPU: Number of seeds/umbel, THSWT: 1000 seed weight; GC: Genotypic correlation.

The number of flowers and seeds per umbel were among the highest direct negative contributors to seed yield. In this study the number of seeds per umbel had perfect correlation (r=1.000), which was the result of its high direct negative effect and high negative indirect influence through bolting period and flower stalk diameter and its positive indirect influence through flowering period, umbel size and thousand seed weight. Similar results were obtained by Shasha'a et al. (1973), who found that the number of flowers/umbel had a negative direct influence on seed yield in onion cultivars. Considering only the number of flowers/umbel in selection programmes was not important in onion improvement for seed yield as its effect is indirect through other components

Umbel size exerted a very high direct influence on seed yield and also showed a moderately high positive indirect effect via bolting period, flower stalk diameter, number of flower stalk/plant and thousand seed weight. Similarly to these results, Prats Perez et al. (1996) found that umbel diameter had a high direct positive effect on seed yield. It is very important to consider this character in improving onion seed yield.

The number of flower stalks/plant had a high direct effect (1.17) and an indirect positive effect on yield through flowering period, umbel size and thousand seed weight, while it had a high negative effect through flower stalk diameter, flower stalks height, bolting period and number of seeds and flowers/umbel. Most of the yield components were found to contribute positively to seed yield indirectly through flower stalks per plant, the correlation coefficients of which were also moderately high with seed yield and most other components, indicating the importance of this trait in selection programmes for onion seed yield improvement

Thousand seed weight also gave a high indirect negative effect via flowering and bolting period, and number of seeds and flowers/umbel, whereas it had the highest positive indirect effect through stalk diameter and umbel size. It also registered a negligible indirect positive effect through flower stalks/plant. This trait was found to be the most important yield component as its direct and indirect effect was high and positive with yield.

The residual effect was equal to –0.33, which indicated that all the components that could affect onion seed yield were considered in this study. Therefore, based on the information on path coefficient analysis, the selection of onion cultivars for high seed yield needs to be concentrated on number of flower stalks/plant, umbel size, bolting period, thousand seed weight and flower stalk diameter.

Conclusions

A positive genotypic correlation existed between seed yield and the number of flowers and seeds/umbel, the number of flower stalks/plant, 1000 seed weight and umbel size. Since these components had a significant positive association it has practical implications in onion improvement through selection based on these yield-related traits. Other very interesting positive relationships were also obtained between bolting and flowering period, flower stalk height and diameter, umbel size and number of flowers/umbel, flowering period and flower stalk diameter, umbel size and flower stalk height, and umbel size and thousand seed weight. Bolting and flowering period had negative significant associations with yield which clearly indicates that selection for high seed yield should concentrate on genotypes which flower and bolt early.

In general many of the characters were positively or negatively correlated due to mutual association with other characters. However, from path coefficient analysis, the number of seeds and flowers/umbel, flower stalk height and flowering period had a direct negative influence on seed yield in onions, which indicated that their effect is via other components. In contrast, bolting period, umbel size, number of flower stalks/plant, flower stalk diameter and thousand seed weight had a direct positive influence on seed yield. Therefore, efforts need to be concentrated on these parameters for high seed yield and could be used for developing varieties for the fast growing onion industry in the country.

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Short communication

INFLUENCE OF HERBICIDES ON WEED MANAGEMENT IN TRUE POTATO

J. PANDEY, R. SING and A. K. VERMA

DIVISION OF AGRONOMY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI, INDIA

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The results obtained showed that there was severe competition between potato and the predominant weed species *Coronopus didymus, Chenopodium album, Fumaria parviflora, Melilotus indica* and *Spergula arvensis.* Competition by other weed species was nominal. The maximum reduction in tuber yield due to weed competition was 50.5% in 1997–98 and 63.4% in 1998–99. Weed control treatments lowered the weed density and weed biomass and scaled up tuber yield in both the years, but their effect on weed species differed. Metribuzin killed all the *Chenopodium album* plants and gave excellent control of *Coronopus didymus* (94%) and effective control of other weed species. Pendimethalin inhibited the germination of *Chenopodium album,* gave good control of *arvensis* and lowered the density of other weed species. Fluchloralin completely inhibited the germination of *Fumaria parviflora* and gave good control of *Chenopodium album* and *Spergula arvensis,* but was least effective against other weed species.

The highest yield was recorded in the weed-free treatment, which was significantly superior to all other treatments. Hand weeding + earthing up, isoproturon (1.0 kg/ha), metribuzin and pendimethalin caused an identical increase in tuber yield, which was significantly higher than the increase in the rest of the treatments. Atrazine at 0.25 kg ha^{-1} resulted in a higher increase than when applied at 0.5 kg ha^{-1}. Fluchloralin, paraquat and paddy straw mulch boosted up production, but the increase in tuber yield was not significant.

Key words: *Solanum tuberosum*, herbicides, atrazine, isoproturon, metribuzin, pendimethalin, paraquat

Introduction

In India potato (*Solanum tuberosum* L.) occupies approximately 1.33 million ha with a total production of 22.6 million t, but its average productivity is only 16 t ha^{-1} against a potential of 40 t ha^{-1}. There are several factors that limit its productivity. A major one is severe weed infestation in the early crop growth stages. Weeds ensconce the potato habitat owing to the wide inter- and intra-row spacing, and the slow emergence and growth in the early stage, growing luxuriantly and divesting the crop of a considerable amount of nutrients vital for early crop establishment and growth, thereby resulting in poor crop growth and lower tuber yield (Datta et al., 1969). Crop yield losses due to weeds were estimated to be 40% by Thakral et al. (1989). It is therefore imperative to control the weeds in the early growth stages to ensure a better crop growth environment. Herbicides have been reported to eliminate the competition in the early crop growth stages and to boost crop yields. However, little information is available on the efficacy of promising herbicides in true potato. Hence, the present investigation was undertaken to assess the effect of herbicides on weeds and tuber yield.

Materials and methods

Site and soil

An experiment was conducted at the Indian Agricultural Research Institute, New Delhi (28°38' N latitude, 77°11' E longitude) during the winter season (Oct. to Feb.) of 1997–98 and 1998–99. The soil of the experimental plot was sandy loam with pH 8.0–8.2, organic C 0.53 to 0.65%, available P and K 10.0 and 2.50 kg ha^{-1} in 1997 and 11.5 and 2.52 kg ha^{-1} in 1998, respectively.

Experimental design and treatments

The experiment included twelve treatments (Table 1) and was conducted in a randomised block design with three replications. The crop received 150 kg N ha^{-1} as urea, 14 kg P ha^{-1} as single superphosphate and 80 kg K ha^{-1} as muriate of potash. The full dose of P and K was applied at planting, while nitrogen was given in three splits, half at planting and the remaining half at the first and second irrigations, 15 and 30 days after planting. Crop variety TPS 1/67, with a tuber size of approximately 40 g per tuber, was planted with a spacing of 45 cm × 10 cm on 25 Oct. in both years. The tubers were planted on ridges.

Field techniques

Except paraquat, all the other herbicide treatments were applied one day after planting with the help of a knapsack sprayer fitted with a flat fan nozzel, using a spray volume of 600 litres of water/ha. Paraquat was sprayed when 5% of the potato plants had emerged in both years. Hand weeding and earthing up were done 35 days after planting.

Results and discussion

Effect of treatments on weeds

Competition between potato and *Coronopus didymus* (37 plants m^{-2}), *Melilotus indica* (32 m^{-2}) and *Fumaria parviflora* (13 m^{-2}) was intense. Besides these *Sisymbrium irio, Avena ludoviciana, Convolvulus arvensis* and *Raphanous niger* also competed, but competition by these species was nominal as their population per square metre was extremely low. The competition effect was markedly reduced by weed control treatments, as is evident from the significant decrease in population and dry matter accumulation (Table 1). In the weed-free treatment the field was kept free of weeds by frequent removal, so there was no build-up of the weed population and weed biomass. However, in the normal practice of hand weeding + earthing up the weeds germinated, grew vigorously and accumulated considerable amounts of dry matter (Table 2). The weed control treatments had different effects on each weed species, though they produced the same decrease in both years. Metribuzin killed all the plants of *Chenopodium album* and gave excellent control of *Coronopus didymus* (94%) and good control of *Spergula arvensis, Fumaria parviflora* and other weed species. The effect of pendimethalin on *Chenopodium album* was similar to that of metribuzin, completely inhibiting germination. It also gave good control of *Spergula arvensis,* but was not very effective against *Coronopus didymus, Fumaria parviflora* and other weed species. It was least effective against *Melilotus indica*. Fluchloralin was the most potent herbicide against *Fumaria parviflora.* It gave excellent control of *Spergula arvensis* and good control of

Chenopodium album, but had little effect against *Coronopus didymus, Melilotus indica* and other weed species. Atrazine at 0.5 kg ha^{-1} efficiently controlled *Chenopodium album, Spergula arvensis* and *Fumaria parviflora* (84–87%), but gave poor control of *Coronopus didymus* and *Melilotus indica*. At the lower dose of 0.25 kg ha^{-1} it failed to control *Melilotus indica*, the population of which tended to increase. This may be due to the elimination of competition from other weeds, favouring higher germination. Isoproturon failed to appreciably reduce the population of *Fumaria parviflora*, but gave good control of *Chenopodium album* and *Coronopus didymus* (Table 2). It caused a marginal decrease in the *Spergula arvensis* population and a marked decrease in the *Melilotus indica* population. Paraquat gave reasonably good control of *Melilotus indica* and *Spergula arvensis* but poor control of other weed species. This may be because by the time it was sprayed these weeds had germinated, while others emerged after its application. Paddy straw mulch suppressed the weed growth for 20 days, after which the weeds grew fast and competed with the crop. The differential behaviour of the herbicides may be ascribed to their selectivity against weed species.

Tuber yield

Tuber productivity was not alike in the two years (Table 2). In the 1997–98 season productivity was higher than in 1998–99. The higher yield in 1997–98 may be attributed to the proper soil-air-water balance during the bulking stage owing to the regulated water supply, favourable temperature (20.7°C max and 5.5°C min) and more sunshine hours (4.7 h), while the lower yield in 1998–99 may be due to the imbalance in the soil-air-moisture interface owing to rainfall (15 mm), unfavourable temperature (18°C max and 7.7°C min) and cloudy days (3.7 h sunshine).

Table 1
Effect of treatments on weed species (60 days after planting)

Treatments	Dose a.i. kg/ha	Weed species (%) decrease				
		C. didymus	*M. indica*	*Ch. album*	*S. arvensis*	*F. parviflora*
1. Weedy check	–					
2. Weed-free	–	100	100	100	100	100
3. Hand weeding+earthing up	–	94	86	80	100	65
4. Atrazine	0.500	32	50	84	82	84
5. Atrazine	0.250	+12	+18	58	63	62
6. Metribuzin	0.500	94	55	100	72	65
7. Pendimethalin	1.000	43	18	100	85	43
8. Fluchloralin	1.000	24	43	74	87	100
9. Isoproturon	1.000	50	67	66	25	+31
10. Isoproturon	0.500	19	53	44	20	16
11. Paraquat	1.000	30	56	+38	60	22
12. Paddy straw mulch	–	17	59	4	80	28

C: *Coronopus*; M: *Melilotus*; Ch: *Chenopodium*; S: *Spergula*; F: *Fumaria;* + = increase

Table 2

Effect of treatments on weeds and tuber yield

Treatments	Dose a.i. kg/ha	Tuber yield (q/ha)		Weed population No./0.25 m^2*		Weed dry wt. g/0.25 m^2*	
		1997–98	1998–99	1997–98	1998–99	1997–98	1998–99
1. Weedy check	–	203.12	123.12	6.25(156)	6.50(16)	7.95(23)	8.32(28)
2. Weed free	–	410.42	336.5	0.71	0.71	0.71	0.71
3. Hand weeding + earthing up	–	332.42	313.75	2.86(31)	2.68(28)	2.03(1.4)	2.42(21)
4. Atrazine	0.500	261.25	214.97	4.65(87)	5.03(101)	5.45(11.9)	5.55(12.0)
5. Atrazine	0.250	293.25	279.12	5.48(120)	5.45(119)	5.86(13.8)	6.10(14)
6. Metribuzin	0.500	316.5	311.25	3.03(35)	3.51(50)	4.13(6.8)	4.78(9.0)
7. Pendimethalin	1.000	332.05	306.25	3.78(59)	4.54(82)	4.50(9.6)	5.54(12)
8. Fluchloralin	1.000	282.25	294.58	4.16(69)	4.73(89)	5.05(10.2)	5.72(13)
9. Isoproturon	1.000	346.87	277.08	4.76(91)	5.33(114)	4.6(8.5)	5.26(11)
10. Isoproturon	0.500	202.08	182.50	4.93(97)	5.48(119)	5.25(11.0)	5.86(14)
11. Paraquat	1.000	252.08	139.15	5.03(101)	5.54(120)	5.53(12.2)	5.99(14)
12. Paddy straw mulch	–	268.75	151.25	4.93(96)	5.63(122)	6.29(15.8)	6.54(17)
S Em +	–	9.08	6.39	0.101	0.083	0.082	0.048
CD at 5%		26.64	18.74	0.30	0.24	0.25	0.14

*: 60 Days after transplanting; Figures in parenthesis are number of weeds m^{-2} and weed dry weight q ha^{-1}

In both years keeping the crop free of weed competition (weed-free) resulted in the highest yield, which was significantly superior to other treatments. The effect of isoproturon 1.0 kg ha^{-1}, metribuzin and pendimethalin was similar in both years, resulting in a significant increase in yield, as compared to the rest of the treatments. The increase in tuber yield in these treatments may be attributed to the effective control of weeds and better crop growth. The lower efficiency of fluchloralin may be attributed to volatilisation losses as it was not incorporated in the soil. In the paraquat-treated plots, weeds that emerged after its application grew vigorously and competed with the crop. Owing to this the increase in yield was not conspicuous. Metribuzin and hand weeding effectively controlled weeds in potato and boosted up the tuber yield significantly. Similar results were earlier reported by Pandey and Sukla (1985). A marked increase in tuber yield and a drastic decrease in weed density and weed biomass were observed by Yaduraju et al. (1998). Akhade et al. (1984) recommended atrazine at 0.3 to 0.5 kg ha^{-1} for the effective control of weeds and the augmentation of tuber productivity. The lower efficacy of paraquat may be ascribed to the luxuriant growth of *Fumaria parviflora*, which emerged after its application and quelled crop growth and tuber yield.

The present study suggests that to achieve higher tuber yields, the crop must be kept free of weed competition from the early crop growth stages. Herbicides effectively control the weeds and augment tuber productivity in a similar manner to the common practice of hand weeding + earthing up. Metribuzin, isoproturon and pendimethalin brought about a marked increase in crop yield.

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Short communication

STUDIES ON INTERCROPPING POTATO WITH FENUGREEK

R. PRASAD, R. SINGH, S. SING and M. PAL

DIVISION OF AGRONOMY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI, INDIA

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A field study conducted for two years at the Indian Agricultural Research Institute, New Delhi showed that intercropping potato with fenugreek is highly profitable and provides some in-season income to the potato growers. It also serves as an insurance against complete loss of income when the potato prices crash in the market.

Key words: potato, fenugreek, intercropping, herb

Introduction

Growing potatoes in Asia is always a gamble. When the potato acreage is large and yields are high, the prices crash and many potato growers leave thousands of acres unharvested. Cold storage facilities are not adequate and are highly costly and the harvested produce has to be sold at a very low price. In the river plains of northern India, the major potato-producing area, the crop is generally planted in October–November and harvested in February–March requiring 140-150 days. During this period the potato growers have no income, while the growers of leafy vegetables such as spinach (*Spinacia oleracea* L.) start harvesting after 60 days and have a steady source of income for 6 months or so by taking cuts each month.

Fenugreek (*Trigonella foenum-graecum* L.) is a herb widely grown in India as a leafy vegetable as well as for its grain, which is used as a spice as well as in the formulation of herbal medicines (Singh et al., 1996). In the present study fenugreek was grown as an intercrop in potato and the results obtained were quite encouraging. The choice of fenugreek was made because it is a legume and has a deep rooting system that does not interfere with the potato-feeding soil layer.

Materials and methods

The field experiment was conducted during the crop years 1998–99 and 1999–2000 (July-June of the succeeding year) on sandy loam soil of pH 8.1, having 19.2 kg ha^{-1} 0.5 M $NaHCO_3$-extractable P, 227 kg ha^{-1} 1M NH_4OAC-extractable K and 176 kg ha^{-1} alkaline permanganate-extractable N, determined as per the procedures described by Prasad (1998). The potato var. Kufri Badshah was planted at a spacing of 20 cm on ridges 60 cm apart in the first week of November and harvested in the second/third week of March. Fenugreek, var. Kasuir, was sown in the fourth week of November, after the germination of the potato tubers, in furrows in between the potato ridges and harvested for grain in the last week of March. There were 4 crop combination treatments, namely, 1. Sole crop of potato, 2. Sole crop of fenugreek, 3. Fenugreek planted in alternate rows in potato (1:1) and 4. Fenugreek planted in furrows after each 2 rows of potato.

Fenugreek had two cutting treatments, namely, no or one cut for green foliage as a vegetable at 70 days after sowing before the final harvest for grain. Thus in all there were seven treatments (Table 1), which were replicated four times in a completely randomized block design. The potato crop received 60 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} as ordinary super phosphate and 100 kg K_2O ha^{-1} as muriate of potash at seeding and 60 kg N ha^{-1} at earthing up in the second week of January. No additional fertilizer was applied for fenugreek.

Results and discussion

Data on the green foliage and grain yield of fenugreek, the tuber yield of potato and the potato equivalents are given in Table 1. Potato equivalents were calculated on the basis of the prices for fenugreek leafy foliage and grain and for potato tubers, which are given in the footnote to Table 1.

Table 1
Effect of intercropping potato with fenugreek

Treatment	FG foliage		FG grain		Potato tubers		Potato equivalents[4]	
	t ha^{-1}							
	I	II	I	II	I	II	I	II
Sole potato	–	–	–	–	22.8	31.9	22.9	31.9
Sole FG	–	–	1.9	2.2	–	–	13.0	14.6
Sole FG*	2.7[2]	2.5	1.7	1.6	–	–	13.7	19.2
Potato + FG (1:1)[3]	–	–	0.3	0.9	25.6	33.8	27.9	39.8
Potato + FG (1:1)[1]	0.7	0.7	0.3	0.6	19.0	32.0	28.8	38.2
Potato + FG (1:2)	–	–	1.1	0.6	25.2	29.9	28.0	34.2
Potato + FG (1:2)[1]	0.3	0.4	0.3	0.4	23.7	29.8	26.9	33.7
LSD (0.05)	0.25	0.26	0.20	0.24	0.9	4.2	1.3	3.8

I. 1998–99; II. 1999–2000;1: one cut for foliage; 2. Fresh weight; 3. Row ratio 1:1 – one row of fenugreek to every row of potato; 2:1 – one row of fenugreek after two rows of potato; 4. Price of potato tubers, Indian Rupees (IR) 1500 t^{-1}; FG green vegetable IR 5000 t^{-1}, FG grain IR 10,000 t^{-1} (1 US $ = 45.80 IR)

In the first year of study the cultivation of fenugreek as an intercrop, i.e. in a 1:1 or 2:1 ratio, increased the potato yield by about 2.5 t ha^{-1} when no cut of fenugreek as a fresh vegetable was made. Taking a cut of fenugreek as a fresh vegetable reduced the potato tuber yield as compared to not cut. This could be due to the protection from low night temperatures provided by fenugreek to the potato plants; the temperatures in January can go as low as 3°C during the nights. In the second year of the study no such effect was observed.

The grain yield of fenugreek in the sole plots was reduced when a cut for fresh vegetable was taken in both years of the study. However, within the treatments involving a cut, the weight of fresh vegetable and grain from fenugreek was significantly higher in the sole crop than in the 1:1 potato:fenugreek intercrop, which in turn gave significantly more than the 2:1 potato:fenugreek intercrop.

Potato equivalents for the different treatments were calculated on the basis of prevailing market prices (given as a footnote to Table 1) and the highest values in both years were obtained for the potato:fenugreek 1:1 ratio intercrop, followed closely by the 2:1 intercropping system. In both years of the study the potato equivalent for the potato:fenugreek 1:1 ratio was significantly superior to the sole crop of potato or fenugreek.

The intercropping of potato with fenugreek is thus a highly remunerative cropping system with an in-season income and an insurance to the potato growers against complete financial loss and is recommended for small and marginal farmers in developing countries.

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Short communication

EFFECT OF GRADED LEVELS OF NITROGEN TO MAIN CROP ON THE PERFORMANCE OF INTERCROPPED LEGUMES GROWN WITH AND WITHOUT FERTILIZERS

O. P. SHARMA and A. K. GUPTA

DEPARTMENT OF AGRONOMY, SKN COLLEGE OF AGRICULTURE, RAJASTHAN AGRICULTURAL UNIVERSITY, CAMPUS: JOBNER, RAJASTHAN, INDIA

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The results of a field study revealed that the application of fertilizers to the companion crop in a millet/legume intercropping system is essential to optimize the yield of the legume component. Supplying nutrients to the main crop alone tended to decrease the productivity of the companion crop, probably because of shading as a result of overgrowth of the main crop. The highest contents of N and P in the grain and straw of the intercrop were recorded with 100% of the recommended dose to both the component crops ($M_{100}I_{100}$). However, the uptake of nutrients was highest from the plots receiving 50 and 100% of the recommended dose to the main and companion crop, respectively ($M_{50}I_{100}$). Higher uptake was due to the fact that the yields increased to a greater extent than the nutrient concentrations.

Key words: clusterbean, cowpea, intercropping, mungbean, nitrogen, pearlmillet

Introduction

The malady of protein malnutrition in semi-arid tropics (SAT) can be reduced to a considerable extent by increasing the production of grain legumes. With the emphasis on cereal production in the SAT, there is less scope for increasing the area under grain legumes. One of the possible ways of increasing their production is the inclusion of grain legumes in intercropping. Of late, research has also generated increasing evidence that substantial yield advantages can be achieved from cereal-legume intercropping compared to sole cropping (Ofori and Stern, 1987; Clark and Myers, 1994; Gupta and Rai, 1999). However, there is meagre information about the fertilizer requirements of intercropping systems. The available recommendations are often restricted to the main crop only, assuming that the legume component can fulfil its own requirements. In such situations, the yields of both the component crops are low compared to the sole crop, though the overall advantage over the pure crop is high (Willey et al., 1981). Roy and Barun (1983) pointed out that the potential of an intercropping system could be increased by supplying the recommended dose of fertilizers to both the component crops. Intercropping systems involving pearlmillet (*Pennisetum glaucum*) as a main crop together with mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*) and clusterbean (*Cyamopsis tetragonoloba*) have long been practised mainly as a strategy to minimize risk, so the yield from the intercrop is considered just as a bonus. The question was

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therefore raised of whether the yield of an intercrop could be improved by supplying the recommended amounts of nutrients separately to the intercrop. The present paper describes the effects of nutrients applied to the main crop and companion crops on the yield and NP nutrition of three different legumes intercropped in pearlmillet.

Materials and methods

The experiment was conducted at the Agronomy Farm of the SKN College of Agriculture, Jobner (26°05' N, 75°28' E) for two consecutive years. The climate of the region is hot and semi-arid. The values of T_{mean}, PET and annual average rainfall are >30°C, 1800 mm and 450 mm, respectively. The rainfall is seasonal in character and its distribution is erratic. The soil of the experimental site was a loamy sand with a pH value of 7.9, poor organic carbon (0.16%) and low water retentivity (171 mm/m depth). All these necessitate tapping the ground water for irrigation at the most critical stages of plant growth. The amounts of native available N, P and K in the soil were 143, 7.1 and 142 kg ha^{-1}, respectively. Traditionally, the farmers opt for pearlmillet (PM) in the rainy season in order to obtain food grain for the family and stover for the livestock. The sole crop of PM is conventionally sown in lines 45 cm apart. In the present experiment, the improved method of Sharma and Gupta (2000) was adopted, wherein the PM was sown in paired rows with 30/60 cm spacings to accommodate an additional row of a legume crop. Thus one row of cowpea (CP) or mungbean (MB) or clusterbean (CB) was inserted in the gap of 60 cm so formed in between the 2 adjacent paired rows of PM. In this way, intercropping allowed an extra population of grain legumes without bringing a separate area under them. The treatment consisted of applying fertilizer to either the main crop alone or to the main crop and the intercrop. The 7 combinations included a control (without fertilizer) and different proportions of the recommended doses of nitrogen. For PM and legumes the recommended quantities of nitrogen were 60 and 15 kg ha^{-1}, respectively. Except for the control plots of intercrop each plot was given a uniform basal dose of phosphorus at 30 kg P_2O_5 ha^{-1}. In the treatment allocation M denotes the main crop of PM and I the intercrop (clusterbean, cowpea or mungbean). $M_{100}I_{100}$ plots were those where the main crop received 100% dose of nitrogen and the intercrop also received the full dose of N. The other fertility combinations were $M_{100}I_0$, $M_{75}I_{100}$, $M_{75}I_0$, $M_{50}I_{100}$, $M_{50}I_0$ and M_0I_0 (Control). The experiment was conducted under rainfed conditions. The amount of rainfall received in 1995 and 1996 was 555 and 690 mm, respectively, but the distribution in 1995 was erratic and it was received on rare occasions. The crops were harvested at maturity and the grain was separated manually. The grain yield recorded in kg $plot^{-1}$ was converted to kg ha^{-1}. Standard analytical techniques were followed to determine the N and P concentrations in the plant material. The protein content in the grain was calculated by multiplying the N content by a factor of 6.25.

Results and discussion

Considerable differences were obtained in the yield levels of the different legumes, which could be attributed to their genetic character and their ability to react with the environment. The results also showed significant differences in the yields of the crops in the two seasons of the experiment. An earlier paper (Sharma and Gupta, 2000) reported that favourable weather in 1996 promoted the growth of the main crop, which resulted in partial shading of the intercropped legumes, resulting in lower yields than those in the previous year. Large variations in the yield levels due to seasonal variability were also observed by Chaurasia and Sharma (1999).

The application of nitrogen to the main crop slightly improved the grain and straw yields of the intercrop legumes over the control (Tables 1,2). The magnitude of increase in the mean grain yield varied between 2% (cowpea) and 4% (mungbean), which was statistically not significant in either year. It was interesting to note that the yields of legumes decreased when the level of nitrogen to the main crop increased beyond 50% of the recommended dose. The reason for the decrease in the yields of legumes from the plots receiving $M_{100}I_0$ compared to that from $M_{50}I_0$ could be shading due to the overgrowth of the main crop, which might have curtailed the rate of photosynthesis in the intercrops.

Table 1

Effect of fertilizer application to main and intercrop on the grain yield, NP concentration and their uptake by intercrops

Treatment	Grain yield (kg ha^{-1})		N content (mg g^{-1})		Protein (%)		P content (mg g^{-1})		N uptake (kg ha^{-1})		P uptake (kg ha^{-1})	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Crop/Fertility levels												
A) Cowpea intercrop												
M_0I_0	242	185	34.4	33.4	21.5	20.9	4.3	4.2	8.4	6.2	1.1	0.8
$M_{50}I_0$	258	202	34.9	34.0	21.8	21.2	4.6	4.4	9.0	6.9	1.2	0.9
$M_{50}I_{100}$	340	258	38.2	36.3	23.9	22.7	5.5	5.3	13.0	9.4	1.9	1.4
$M_{75}I_0$	253	196	37.0	34.6	23.1	21.6	4.8	4.7	9.3	6.8	1.2	0.9
$M_{75}I_{100}$	334	249	39.1	37.3	24.5	23.3	5.8	5.7	13.2	9.3	1.9	1.4
$M_{100}I_0$	229	175	37.5	36.2	23.4	22.6	5.0	4.9	8.6	6.3	1.2	0.9
$M_{100}I_{100}$	298	236	41.0	37.3	25.6	23.3	6.1	6.1	12.2	8.8	1.8	1.4
S Em ±	18	13	1.5	1.2	0.9	0.7	0.04	0.1	0.9	0.9	0.1	0.1
LSD (5%)	52	38	NS	NS	NS	NS	0.1	0.3	2.6	2.6	0.3	0.2
B) Mungbean intercrop												
M_0I_0	248	237	35.3	34.1	22.1	21.3	3.8	3.7	8.8	8.1	0.9	0.9
$M_{50}I_0$	272	260	35.7	35.2	22.3	22.0	3.9	3.8	9.7	9.2	1.1	1.0
$M_{50}I_{100}$	364	317	37.9	36.1	23.7	22.6	4.2	4.2	13.9	11.5	1.5	1.3
$M_{75}I_0$	254	247	36.4	35.5	22.7	22.2	4.0	3.9	9.3	8.8	1.0	1.0
$M_{75}I_{100}$	353	304	39.8	37.6	24.9	23.5	4.3	4.2	14.0	11.4	1.5	1.3
$M_{100}I_0$	252	238	37.1	36.5	23.2	22.8	4.1	4.0	9.4	8.7	1.0	1.0
$M_{100}I_{100}$	345	286	40.7	38.2	25.4	23.9	4.4	4.3	14.1	10.9	1.5	1.2
S Em ±	25	18	1.3	0.6	0.8	0.4	0.1	0.1	1.2	0.6	0.1	0.1
LSD (5%)	74	52	NS	NS	NS	1.1	0.2	0.1	3.6	1.8	0.3	0.2
C) Clusterbean intercrop												
M_0I_0	354	181	32.3	30.0	20.2	18.7	3.3	3.2	11.4	5.4	1.2	0.6
$M_{50}I_0$	372	208	33.8	31.1	21.1	19.4	3.4	3.3	12.5	6.5	1.3	0.7
$M_{50}I_{100}$	465	251	35.6	33.3	22.2	20.8	3.6	3.5	16.5	8.4	1.7	0.9
$M_{75}I_0$	379	191	34.1	32.9	21.3	20.5	3.4	3.3	12.9	6.3	1.3	0.6
$M_{75}I_{100}$	453	230	36.6	33.8	22.9	21.1	3.7	3.6	16.6	7.8	1.7	0.8
$M_{100}I_0$	332	186	34.4	33.1	21.5	20.7	3.5	3.4	11.4	6.2	1.2	0.6
$M_{100}I_{100}$	428	223	37.4	35.4	23.4	22.1	3.8	3.6	16.1	7.9	1.6	0.8
S Em ±	23	12	1.4	0.8	0.9	0.5	0.1	0.01	1.3	0.3	0.1	0.1
LSD (5%)	68	36	NS	2.3	NS	1.4	0.2	0.04	3.7	0.9	0.3	0.1

Y_1=1995, Y_2=1996, NS=Not significant

Table 2
Effect of fertilizer application to main and intercrop on the straw yield, NP content in straw and nutrient removal by intercrops

Treatment	Straw yield ($kg\ ha^{-1}$)		Nutr. content ($mg\ g^{-1}$) N		Nutr. content ($mg\ g^{-1}$) P		Nutrient uptake ($kg\ ha^{-1}$) N		Nutrient uptake ($kg\ ha^{-1}$) P		Mean* ($kg\ ha^{-1}$)	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Crop/Fertility levels												
A) Cowpea intercrop												
M_0I_0	669	547	12.9	12.4	2.2	2.1	8.6	6.8	1.4	1.1	15.0	2.2
$M_{50}I_0$	799	625	13.4	12.7	2.4	2.3	10.7	8.0	1.9	1.4	17.3	2.7
$M_{50}I_{100}$	1025	757	13.8	13.6	2.9	2.9	14.1	10.3	3.0	2.2	23.4	4.2
$M_{75}I_0$	814	571	14.1	12.7	2.7	2.5	11.5	7.3	2.2	1.4	17.5	2.9
$M_{75}I_{100}$	920	744	14.6	13.9	3.1	3.1	13.4	10.4	2.8	2.3	23.2	4.3
$M_{100}I_0$	694	584	14.2	13.1	2.8	2.7	9.9	7.7	2.0	1.6	16.3	2.8
$M_{100}I_{100}$	919	705	14.5	14.1	3.4	3.2	13.4	9.9	3.1	2.3	22.2	4.3
S Em ±	36	28	0.4	0.3	0.06	0.05	0.6	0.6	0.1	0.1	–	–
LSD (5%)	107	83	1.1	1.0	0.2	0.14	1.7	1.9	0.4	0.3	–	–
B) Mungbean intercrop												
M_0I_0	548	516	14.6	13.7	2.3	1.7	8.0	7.0	1.2	0.9	16.0	2.0
$M_{50}I_0$	546	513	14.9	13.9	2.4	1.8	8.1	7.1	1.3	0.9	17.1	2.1
$M_{50}I_{100}$	704	702	15.5	15.3	2.8	2.3	10.9	10.7	2.0	1.6	23.5	3.2
$M_{75}I_0$	491	514	15.2	15.0	2.5	1.9	7.5	7.7	1.2	1.0	16.6	2.1
$M_{75}I_{100}$	691	671	16.0	15.7	2.8	2.8	11.0	10.5	2.0	1.9	23.5	3.3
$M_{100}I_0$	525	518	15.4	15.1	2.6	2.1	8.1	7.8	1.4	1.1	17.0	2.2
$M_{100}I_{100}$	688	598	16.2	16.1	3.0	2.9	11.1	9.6	2.1	1.8	22.9	3.3
S Em ±	41	34	0.3	0.3	0.04	0.05	0.7	0.6	0.1	0.9	–	–
LSD (5%)	120	99	0.9	1.0	0.14	0.14	2.0	1.8	0.3	2.6	–	–
C) Clusterbean intercrop												
M_0I_0	1005	588	8.8	8.5	1.7	1.7	8.9	5.0	1.8	1.0	15.4	2.3
$M_{50}I_0$	1079	578	8.9	8.7	1.8	1.7	9.7	5.1	1.9	1.0	16.9	2.4
$M_{50}I_{100}$	1362	751	9.8	10.0	1.9	1.9	13.3	7.5	2.6	1.4	22.6	3.3
$M_{75}I_0$	1080	574	9.0	9.8	1.8	1.8	9.8	5.6	2.0	1.0	17.3	2.5
$M_{75}I_{100}$	1314	699	10.0	10.8	2.0	1.9	13.1	7.5	2.6	1.4	22.5	3.2
$M_{100}I_0$	1022	558	9.2	9.8	1.9	1.9	9.4	5.5	1.9	1.0	16.3	2.4
$M_{100}I_{100}$	1322	611	10.2	11.1	2.2	2.1	13.5	6.8	2.9	1.3	22.2	3.3
S Em ±	73	36	0.1	0.2	0.05	0.02	0.8	0.5	0.2	0.1	–	–
LSD (5%)	214	105	0.4	0.5	0.14	0.06	2.3	1.5	0.5	0.2	–	–

Mean*: Mean of total nutrient removal; Y_1=1995, Y_2=1996, NS= Not significant

Similar results were obtained by Yadav and Yadav (2000), who reported that the reduction in seed yield of clusterbean (companion crop) was greater in mixed stands with tall pearlmillet (main crop) than with medium-statured hybrid pearlmillet. All the intercrops responded well to fertilizer application irrespective of the level of fertilizer to the main crop. The highest yields were recorded at $M_{50}I_{100}$. This suggests that the application of fertilizer to the intercrop component is essential to improve its yields. Yadav et al. (1994) also observed improved yields of cowpea and mungbean grown with pearlmillet when the component crops were fertilized with the recommended level of nutrients. The recommendations of Kwari et al. (1998), suggesting an additional dose of fertilizers to the intercrop in the millet/legume mixture, also corroborate these findings. The nitrogen content in the seeds and straw of different crops did not increase significantly as a result of the nutrient supply to the main crop. The same was true of the protein content in the grains. However, the protein content increased appreciably due to fertilizer supply to the intercrops. Further, the nitrogen and phosphorus contents increased significantly due to the application of 100% of the recommended dose of fertilizer to both the component crops ($M_{100}I_{100}$). The data further revealed that the uptake of N and P was highest from $M_{50}I_{100}$, which was due to the fact that the yields increased to a greater extent than the nutrient concentration.

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Review

INTERCEPTION AND USE OF LIGHT BY SUNFLOWER (*HELIANTHUS ANNUUS* L.)

M. LONG[1], B. FEIL[1] and W. DIEPENBROCK[2]

[1]INSTITUTE OF PLANT SCIENCES, ETH ZURICH, ZURICH, SWITZERLAND
[2]INSTITUT FÜR ACKER- UND PFLANZENBAU, MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, HALLE (SAALE), GERMANY

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Literature on the effects of canopy structure, light environment, water stress, nutritional factors and planting patterns on the interception and use of light by sunflower (*Helianthus annuus* L.) is reviewed. As with other crops, the effectiveness of the canopy to use light depends on the structural and functional distribution of leaf area; however, the sunflower is (almost) unique in that heads and leaves show phototropical movement. The responses of light interception to variations in plant density and row spacing are inconsistent. There is little information available about the effect of row orientation on light use. Field experiments in a large range of latitudes and model approaches are required to understand the effects of environment and planting pattern on the yield performance of sunflower.

Key words: sunflower (*Helianthus annuus* L.), light interception, canopy structure, plant density, row spacing, row orientation, water stress, nutrient stress

The sunflower (*Helianthus annuus* L.) crop excels the other C_3 species in the rate of photosynthesis and the efficiency of photon uptake by the growing leaf canopy, even though it reveals the typical leaf structure of a C_3 plant. The net photosynthesis of the sunflower leaf (65 mg CO_2 dm^{-2} h^{-1} at high irradiation) is close to that of C_4 plants (between 40 and 50 mg CO_2 dm^{-2} h^{-1}), while other C_3 plants regularly reach assimilation rates between 20 and 25 mg CO_2 dm^{-2} h^{-1}. This peculiarity is due to several physiological leaf characteristics including large stomatal conductance, high rubisco activity, efficient electron transport and competitive membrane transport of chloroplasts (reviewed by Diepenbrock and Pasda, 1995). The exceptional photosynthetic ability of single leaves compensates for the canopy structure which is principally unsuited for high productivity, because it is characterized by a horizontal display of mature leaves. Thus, the high photosynthetic potential does not necessarily translate into high radiation use efficiency. On the other hand, heliotropic movements of the leaves enable the plant to absorb more light and thereby increase the photosynthesis of the juvenile stand (Shell and Lang, 1976).

In crop production, light interception (the difference between incoming light and transmitted light, c.f. Daughtry et al., 1992; Gallo et al., 1993) differs

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widely, depending on growth stage and environmental factors, i.e. light environment, water stress, nutritional factors, and planting patterns. A detailed analysis of interception and the use of light in relation to environmental factors and planting patterns is given in this review to better understand the relationship between incoming radiation and sunflower growth. The information could aid crop modelers in constructing an ideotype that can be used by breeders to select for higher yield.

1. Light interception and radiation use efficiency (RUE)

The interception of light depends on the position of the photosynthetic tissue in relation to the light beams. Thus, it is influenced by leaf and canopy structure, e.g. the spatial arrangement of green leaves and other shading organs, especially the head of the sunflower. The absorption of light (the difference between incoming light and transmitted light plus reflected light, c.f. Daughtry et al., 1992; Gallo et al., 1993) largely follows the changes in light interception. Under optimum growing conditions, radiation use efficiency (RUE, the slope of the regression line between the dry matter of the whole plant or plant parts, respectively, and cumulative absorbed light, c.f. Daughtry et al., 1992; Gallo et al., 1993) is determined not only by the canopy structure, but also by the photosynthetic capacity of chlorophyllous organs and the amount of energy for the production of per unit dry matter. The environmental conditions, e.g. the availability of light, nutrients and water, may also alter light interception and RUE.

1.1 Light interception and RUE in sunflowers

According to Cupina et al. (1988), 20% of the incoming light is reflected by the canopy, 55 to 59% is absorbed by the plants, and 20% is transmitted to the soil surface. The RUE of sunflower ranges from 1.3 to 5.0 g MJ^{-1}, depending on the genotype and plant age (Kiniry et al., 1989). Various authors described the seasonal pattern of RUE in sunflower canopies. Gimenez et al. (1994) reported that the RUE was 0.74 and 0.92 g MJ^{-1} during the establishment of the canopy and increased to a maximum of 2.29 g MJ^{-1} during flowering. Trápani et al. (1992), Steer et al. (1993), Hall et al. (1995) and Aufhammer et al. (2000) found that the RUE increased after floret initiation and reached a maximum at anthesis. Possible causes of differences in RUE during the life cycle are summarized in Table 1.

Table 1
Possible causes of variations in radiation use efficiency of a sunflower canopy

Causes	References
High synthesis costs of the oil-rich achenes	Ferraris and Charles-Edwards (1986)
Different chemical conversion efficiency	Rosenthal and Gerik (1991)
Increasing respiratory load	Whitfield et al. (1989), Trápani et al. (1992)
Lower temperatures during establishment	Garcia et al. (1988)
Higher protein concentration in young plants	Hocking and Steer (1989)
Changing photosynthetic capacity of the leaves	Green and Valdyanathan (1986)
Variations in light saturation points	Monteith (1981)
Increasing mutual shading	Trápani et al. (1992)
Appearance of senescent leaves and other photosynthetically inactive plant parts	Monteith (1981), Bange et al. (1997a)

1.2 Effect of canopy structure

Light interception is more dependent on the spatial arrangement and the age-class composition of leaves within the canopy than RUE. In contrast, RUE shows greater dependence on the function structure of the canopy, e.g. rate of photosynthesis, partitioning of photoassimilates, as well as heliotropism.

1.2.1 Light interception

The interception of light in a sunflower canopy is determined by leaf angle, leaf size and internode length. According to Kubota et al. (1994), an optimal canopy for light interception is characterized as follows: the upper layer is composed of small, erect leaves; the leaves become larger and more horizontal towards the stem base. This may also apply to sunflower crops. In fact, Sadras and Villalobos (1993) proposed that a semierect leaf disposition would be desirable to improve light penetration in the canopy and growth of sunflower.

The leaf age structure is also important for light interception. Leaf senescence is associated with changes in the content and distribution pattern of chlorophyll and, thus, influences the canopy's capability to absorb and reflect light. The chlorophyll content of sunflower leaves was found to increase until their full expansion. At the same time, leaf reflectance and transmittance did not change. With increasing senescence the chlorophyll content dropped from 527 to 164 μmol m^{-2}. Leaf absorption declined by 11%, while the transmittance increased by 7% (Masoni et al., 1994).

Leaf area index (LAI, the leaf area per unit ground area) and extinction coefficient (*k*, derived from the nonlinear regression of light interception on LAI and Beer's Law, reviewed by Diepenbrock, 1997) are two parameters that are frequently used to describe the quantitative relationship between canopy structure and light interception. Possibly, there is no exponential relationship between LAI and light interception in sunflower canopies because the canopy structure changes with time (Sadras et al., 1991a). Prior to anthesis, the structure of sunflower canopies shows diurnal variation due to leaf phototropism. During flowering, heads show phototropic movement, whereas during achene filling they remain in a fixed position facing the east.

In contrast, the characteristic of k can reflect the influence of canopy structure on light interception. During the development of a sunflower canopy, k decreases until anthesis and then increases towards physiological maturity. This pattern results from the negative correlation between k and LAI (Rawson et al., 1984; Sadras et al., 1991a). A decreasing proportion of leaves showing phototropic movements during the pre-anthesis period might contribute to changes in k (Lang and Begg, 1979).

1.2.2 Radiation use efficiency (RUE)

Radiation use efficiency is determined by the rate of photosynthesis of leaves or other organs. The photosynthetic rate of a sunflower canopy varies with both the age and the position of the leaf along the stem. The rate of leaf photosynthesis increases to a maximum just prior to full leaf expansion and then declines. It is highest in the uppermost layers of the canopy (English et al., 1979).

Apart from the photosynthetic rate, the relative contribution of photoassimilate to the achene is also important for RUE. The upper leaves are of major importance, because approximately 75% of the assimilate produced in the leaves is translocated; more than 80% of translocated assimilate is partitioned to the head. On the other hand, the lower leaves export only 50% of their photosynthetic products, most of which are stored in stem and root tissues (Diepenbrock and Pasda, 1995).

Since the first study by Darwin and Darwin (1880), numerous investigations of leaf phototropism and its effect on RUE have followed (Shell et al., 1974; Shell and Lang, 1976; Casal and Sadras, 1987). Shell and Lang (1976) estimated that, as a result of leaf phototropism, the additional intercepted light can account for a 20% increase in photosynthesis in sunlit leaves, thereby promoting RUE. Lang and Begg (1979) studied the characteristics of sunflower phototropism and drew the following conclusions: diurnal east-west oscillations of the heads occur at early stages of development, but they cease when the flowers open and anthesis commences. Thereafter heads face east.

1.3 Influence of light environment

A decrease in the red/far-red ratios of sunflower crops enhances the basal leaf senescence (Rousseaux et al., 1996, 1999). Ratios of red to far-red can be modified by changing plant density and row orientation. Plants in narrowly spaced rows or dense populations are exposed to higher red/far-red ratios than those grown in widely spaced rows or sparse populations. Phototropic leaf movement can contribute to the far-red reflection patterns associated with row orientation (Rousseaux et al., 1996).

Light intensity and the position of the sun can influence the extinction coefficient (*k*). Rawson et al. (1984) found that *k* under sunflower canopies differed between high (25.5 MJ day^{-1}) and low (9.5) light intensity. There is diurnal variation in *k*; *k* is at a minimum at noon, irrespective of whether it is clear or cloudy; furthermore, *k* increases as the angle of the sun decreases (Sadras et al., 1991a; Flénet et al., 1996). Likewise, Bange et al. (1997a) reported that sowing date affected sunflower yields due to a correlation between light environment and biomass production. When the light intensity decreased and the proportion of diffuse light increased, dry matter accumulation remained constant, while RUE increased.

The leaf area of sunflower was found to be reduced markedly by shading, which resulted in smaller (Hiroi and Monsi, 1963) or more senescent leaves (Hiroi and Monsi, 1964; Dosio et al., 2000). However, in the experiment of Rawson and Hindmarsh (1983), shading did not have a clear effect on the sunflower canopy structure, because, under low light intensity, the leaf area was maintained at the expense of the leaf weight per unit leaf area. Due to adverse effects of shading on the 1000-achene weight, the achene yield decreased even though RUE increased (Villalobos et al., 1992; Andrade, 1995; Andrade and Ferreiro, 1996).

1.4 Morphological and physiological adjustment to water stress

Water shortage can affect RUE (Begg and Turner, 1976). Diurnal variation in the extent of wilting of sunflower leaves increases the RUE, since wilting reduces the interception of light around midday (Fredeen et al., 1991; Maury et al., 2000). The limited effect of water stress on photosynthesis can be attributed to the adjustment of LAI rather than of leaf water conductance and photosynthetic rate (Connor et al., 1985; Connor and Hall, 1997). Physiological adjustments in water-stressed plants change with canopy development. Before anthesis, water stress reduces leaf area, thereby decreasing light interception and transpiration (Sadras et al., 1991b). After anthesis, transpiration is controlled both by lower light interception (by more extensive leaf senescence) and less water conductance of the leaf canopy (Connor and Sadras, 1992). During achene filling, water stress leads to an immediate decrease in gross CO_2 assimilation, which can be attributed to a rapid loss of leaf area and a decreased RUE (Whitfield et al., 1989).

1.5 Nutritional factors

The availability of nitrogen (N) may affect achene yield, dry matter production and RUE. The major effect of N on the early growth of sunflower plants is mediated by the leaf area. Nitrogen deficiency decreases the number and area of individual leaves while leaf duration remains unchanged. This response causes reductions in light interception (Steer and Hocking, 1983; Sadras et al., 1991b; Steer et al., 1993; Bange et al., 1997b, c; Scheiner and Lavado, 1999; Trápani et al., 1999; Rousseaux et al., 2000). Sarmah et al. (1992) demonstrated that LAI, achene yield and RUE increased significantly as the N rates increased from 0 to 100 kg N ha^{-1}. Joel et al. (1997) found that LAI and RUE of sunflower plants decreased by more than 30% under N deficiency. Gimenez et al. (1994) showed that only a small part of the observed RUE response to N deficiency was attributable to a decreased rate of photosynthesis. Specific leaf N (SLN), the amount of N per unit green leaf area, is often used to describe the influence of the N concentration in the leaf on crop RUE (Muchow, 1988). The RUE of sunflower crops is positively correlated with canopy SLN (Hall et al., 1995). This may explain the decrease in RUE during achene filling.

In various studies, neither achene yield nor achene oil concentration were found to be influenced by the rate of phosphorus (P) application (Rodriguez et al., 1998). Colomb et al. (1995) confirmed this but observed that both light interception and RUE increased significantly with more than 35 kg P ha^{-1}.

2. Effect of plant density, row spacing and row orientation on light interception and RUE

Planting patterns may affect the canopy structure, and thus, the partitioning of light between the plant and soil surfaces. Due to interplant competition for light and other yield-determining factors, the achene yield of individual plants will decrease as plant density increases. Even though mutual shading of the plants increases (Tenebe et al., 1996), Hiroi and Monsi (1966) found the plant density had no effect on the extinction coefficient (k) of sunflower crops. Yield responses to increasing plant density are inconsistent (Miller et al., 1984; Wade and Foreman, 1988; Gubbels and Dedio, 1990; Blamey et al., 1997; Reddy et al., 1997; Long, 1999; Diepenbrock et al., 2001), probably indicating that the optimum plant density depends on the environment and cultivar (Prunty, 1981).

Various experiments indicate that smaller distances between the rows result in greater interception of light (Gubbels and Dedio, 1988; Zaffaroni and Schneiter, 1989; Flénet et al., 1996; Blamey et al., 1997). Flénet et al. (1996) found that k decreased linearly as row spacing increased. The achene yield responses to varied row spacings are inconsistent (Long, 1999; Diepenbrock et al., 2001); Robinson et al. (1982) suggested that phototropic movement and variation in plant height of sunflower crops may help explain this inconsistency.

The effect of row orientation on the sunflower crop is not well understood, probably because only a few experimental data are available

(Robinson, 1975; Diepenbrock et al., 2001). In Minnesota, sunflowers grown in north-south and east-west rows did not differ in achene yield, achene oil concentration, 1000-achene weight or test weight, or in the distribution of achene size (Robinson, 1975). Recently, however, Diepenbrock et al. (2001) reported that the east-west orientation produced higher achene and oil yields than the north-south orientation at a location in central Europe. Some of these conflicting results may be due to the fact that the studies were conducted at different latitudes (Diepenbrock et al., 2001). Some models tried to simulate the response of sunflower to planting patterns, but the results were not very satisfactory (e.g. Connor and Fereres, 1999; Wernecke et al., 2000).

3. Conclusions

Light interception by sunflower canopies depends on the structure of the canopy. The frequently used exponential function between LAI and light interception is probably not applicable to sunflower canopies due to their active phototropism. The radiation use efficiency (RUE) of sunflower crops changes during development and is affected by environmental conditions. Planting patterns affect the transmission of light and the partitioning of light in the canopy. Optimizing plant density, row spacing and row orientation may, therefore, improve the interception of light and RUE, but experiments dealing with the effects of plant density and row spacing on achene yield and other traits gave inconsistent results. The reasons for this remain to be elucidated by further research. Since information on the growth response of sunflower crops to row orientation is still meagre and contradictory, we suggest conducting experiments over a large range of latitudes to create a dataset that can be used by modelers.

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BARBARA HARASZTOS

Manuscripts and editorial correspondence should be addressed to

Acta Agronomica Hungarica
Agricultural Research Institute of the
Hungarian Academy of Sciences
H-2462 Martonvásár, Hungary
Phone: (36-22) 569-521
Fax: (36-22) 460-213
E-mail: actaagr@mail.mgki.hu

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DYNAMICS OF DRY MATTER PRODUCTION, TRANSPIRATION AND PHOSPHORUS UPTAKE OF MAIZE (*ZEA MAYS* L.)

S. SZLOVÁK[1] and Z. ALMÁSI[2]

[1]RESEARCH INSTITUTE FOR FISHERIES, AQUACULTURE AND IRRIGATION, SZARVAS, HUNGARY
[2]SAMUEL TESSEDIK COLLEGE, SZARVAS, HUNGARY

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A two-year pot experiment was carried out in a green-house to investigate the dry matter (DM) accumulation, distribution and redistribution in maize, the transpiration intensity/leaf area and the dry leaf weight. The uptake, distribution and redistribution of phosphorus was also studied. The total dry matter weight (DMW) of aerial plant parts increased up to 108 days after emergence (DAE) and then, with the exception of the grain, decreased to the final harvest. Averaged over two years, the most DM was transported to the grain from the stalk (69.41%) and the least from the leaf-sheath (1.69%). The lowest transpiration intensity calculated per dm^2 $hour^{-1}$ was 0.41 g and the highest 1.35 g. The transpiration intensity calculated per unit weight of dry leaf blades was 0.62 and 2.80 g. In both years the total phosphorus uptake increased in all aboveground plant parts up to the 80^{th} day after emergence and then, with the exception of the grain, decreased to the final harvest. At the end of the growing season the grain stored most (84.85%) of the absorbed phosphorus, averaged over two years.

Key words: maize, dry matter production, transpiration, phosphorus uptake

Introduction

The dry matter (DM) accumulation of the whole maize plant proceeds up to the developmental stage of biological maturity. This DM accumulation time varies for different plant parts since after a certain developmental stage a significant part of the carbohydrate stored in the organs is translocated to the developing grain.

Among the three most important macronutrients used in maize production, N is important in the vegetative phase of maize development, and P in the generative phase. Even if there is sufficient P in the soil its availability may be influenced by several factors, such as soil pH, water supply (Debreczeni and Debreczeni, 1983), N content (Szlovák, 1995a), etc. The phosphorus uptake by plants growing in the soil is also affected by the P absorption characteristics of the roots (Barber, 1980). The root growth, mass flow and especially diffusion are important in the P uptake of maize growing on soil (Barber and Olson, 1969). According to Bergmann and Neubert (1976) 91% of plant P is taken by diffusion. Besides morphological and physiological root parameters, the chemical effect of the roots on the soil environment may be involved in P uptake processes from soils (McLachlan, 1976). There are substantial differences in the amount of phosphorus uptake not only between different plant species but also within the same species, for example, between maize hybrids (Szlovák, 1995b).

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Materials and methods

In both years the maize seeds were planted on 7th May in 20×25 cm white enamel-painted, modified Mitcherlich pots containing 6 kg air-dry alluvial meadow surface soil from Szarvas. The maximum water-holding capacity of the soil was determined in the laboratory as 49.7% (expressed as a weight percentage of absolutely dry soil). The other main characteristics of the soil used in the experiment were: pH(H_2O): 5.95, pH(KCl): 5.65, total salt: 0.07%, humus: 2.17%, total N: 0.21%. Available P and K, determined by the methods outlined by Egner et al. (1960), were 36.3 and 179.4 ppm, respectively; soil plasticity index according to Arany: 46.4.

The soil on which the hybrid Pioner MSC 3780 maize plants developed was watered daily to 70% of its maximum water-holding capacity.

The active ingredients of the fertilizers per pot were as follows: N: 0.84 g (ammonium-nitrate), P: 0.52 g (superphosphate), K: 1.00 g (potassium chloride).

Five seeds were sown in each pot. After emergence the plants were thinned to one per pot. There were 7 replicates in the first year and 10 in the second.

Plants were harvested on 16 occasions in the first year and 10 in the second. The stalk and tassel were weighed and analysed together in the first year and separately in the second. In both years the ear-shank was included with the husks. At harvest the roots were washed free of soil. After separation, the plant parts were dried at an oven temperature of 60°C until constant weight was achieved.

The leaf area was calculated by using the method of McKee (1964). In both years the transpiration intensity was calculated for unit leaf area and for unit dry weight of leaf-blades.

The plant parts were analysed spectrophotometrically for P by the method of Tamm et al. (1968) following digestion with sulphuric acid and hydrogen peroxide.

Results and discussion

Dry matter accumulation and translocation

Figure 1 shows the DM accumulation and its distribution among the plant parts during the growth of maize in the first year. The total dry matter weight (DMW) of the plant parts increased up to 28 August, i.e. up to 108 days after emergence (DAE), and then, with the exception of the grain, decreased to the final harvest at 129 DAE. Hanway (1963) also examined the DM dynamics of maize during the growth season, but did not indicate a decline in DM towards the end of the season. This decline in DM was registered in both years of the present experiment. At the peak the DMW of the stalk was 25.15% of the total aerial parts of the plant (Table 1). The lowest value was obtained for the husks (6.40%). Even at this developmental stage the DMW of the grain was the highest (37.88%). The DMW of the roots also decreased from this stage onwards. Most DM was transported from the stalk to the grain (67.55%, Table 2) and least from the leaf sheaths (2.01%). The decrease in root DM (5.43 g) was not taken into account in the calculations. After the sampling at 108 DAE, the total DM increase of the aerial parts was only 15.16 g, while the grain DM increased by as much as 46.13 g. This meant that 30.97 g DM was translocated to the grain. Among the plant parts only the grain DMW increased right up to the final harvest. The final DM distribution percentage among the plant parts is presented in Figure 1.

Table 1

Dry matter distribution (%) in plant parts with the onset of P and DM depletion in the shoot

Year	Days after emergence	Plant parts						
		Stalk	Leaf-sheaths	Leaf-blades	Tassel	Husks	Cob	Grain
First	80	39.16	16.93	27.71		10.35		
	108	25.15	6.81	12.75		6.40	11.00	37.88
Second	80	31.24	12.21	21.29	1.60	11.69		
	107	20.89	6.18	12.32	0.90	6.22	11.21	42.27

Table 2

Dry matter translocation (%) from plant parts to the grain from the time of maximum DMW (28 August)

Year	Plant parts				
	Stalk	Leaf-sheaths	Leaf-blades	Husks	Cob
First	67.55	2.01	5.65	8.94	15.85
Second	71.26	1.35	2.70	10.40	15.28
Average	69.41	1.68	4.18	9.67	15.57

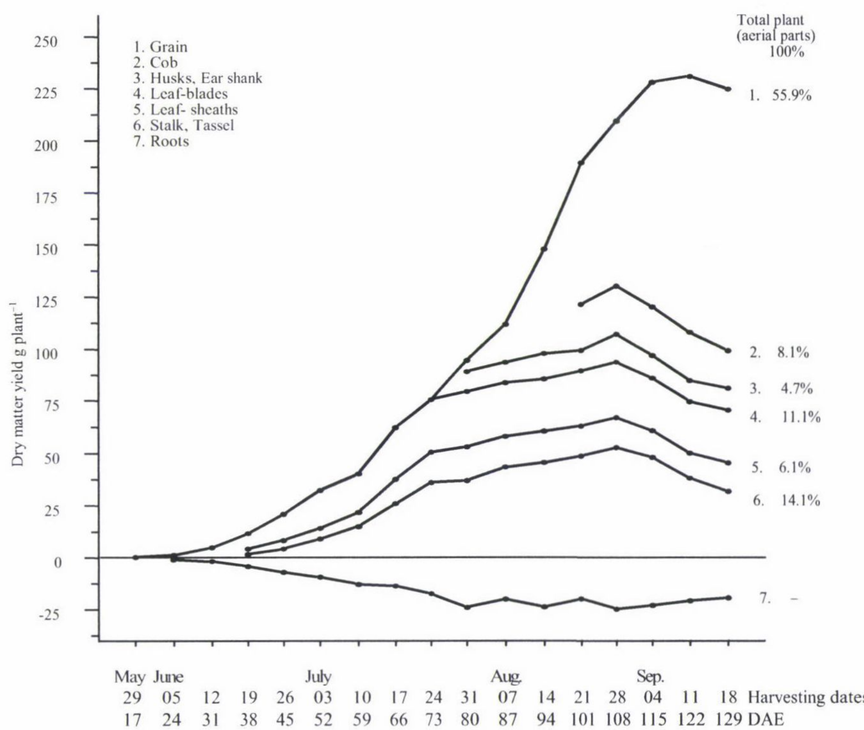

Fig. 1. Dry matter accumulation of maize (First year) (DAE: Days after emergence)

In the second year the DM distribution among the plant parts was similar to that observed in the first year (Fig. 2). The maximum DMW was attained at much the same time as in the previous year (107 DAE), after which the total DMW of the aerial plant parts, with the exception of the grain, steadily declined to the final harvest.

At the start of the steady weight decrease (107 DAE) the DMW of the stalk made up 20.89% of the total DMW of the aerial plant parts (Table 1). The DMW of the other plant parts was close to that registered in the previous year. The DMW percentages of the plant parts at the final harvest were also alike in both years (Fig. 2). If these percentages are compared to the data obtained in an earlier experiment (Szlovák, 1983) relatively small differences are found.

As in the previous year, with the onset of the DMW decline in the aerial parts, with the exception of the grain, there was only a slight increase in total DM (18.12 g). As in the first year most DM (71.27%) was transported from the stalk to the grain (Table 2). The DMW increase in the grain (30.67 g) was significantly lower in the second year, being only 66.49% of that in the previous year. The grain increased by 36.77% of its final weight in the first year and by only 26.64% in the second.

In the second year there may have been certain factors which limited the translocation of DM from other plant parts to the grain. This limitation also manifested itself in the lower final grain yield.

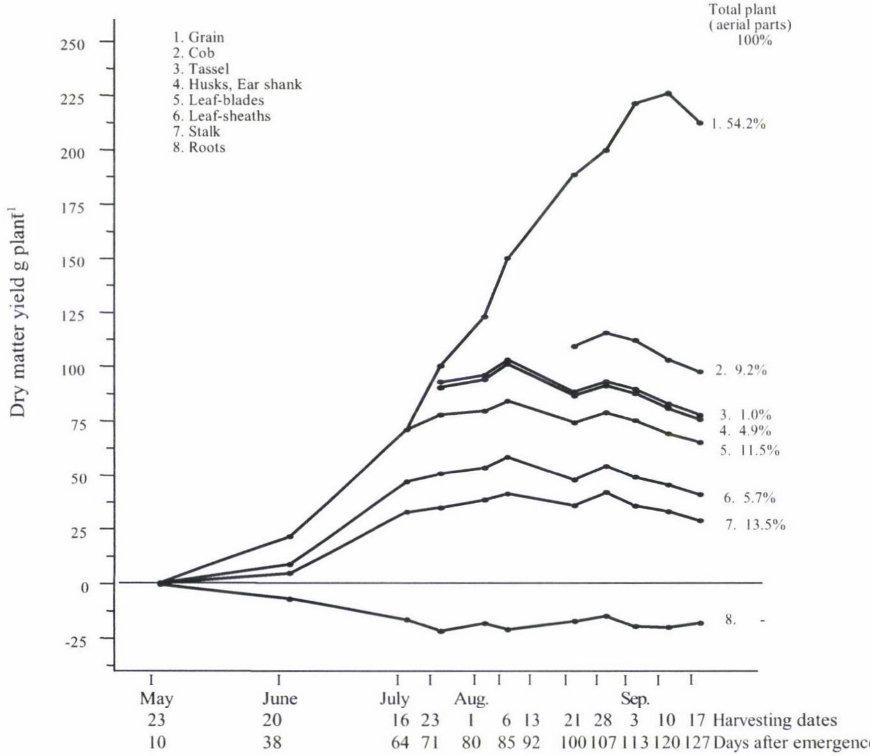

Fig. 2. Dry matter accumulation of maize (Second year)

Dynamics of maize transpiration

The leaf area measurement began on 19th June in the first year and ended on 11th September (Fig. 3). It increased steeply, remained relatively high till 21st August and then declined sharply. The DM of the leaf-blade also increased sharply, but only decreased slightly towards the end of the growth stage. The lowest transpiration intensity calculated per dm^2 $hour^{-1}$ was 0.27 g and the highest 1.35 g. These values were close to those obtained in earlier experiments: 0.38 and 1.62 g (Szlovák, 1972) and 0.17 and 1.32 g (Szlovák, 1974). Similarly, the transpiration intensity calculated per unit weight of dry leaf-blade was 0.62 and 2.80 g in the present case and 0.61 and 2.58 g in an earlier experiment (Szlovák, 1972). Similar values were obtained in the second year of the experiment (Fig. 4), though here the steep increase in leaf area could not be seen, because the first leaf area measurement did not take place until 16th July. The DM of leaf-blade only decreased slightly towards the end of the growth season.

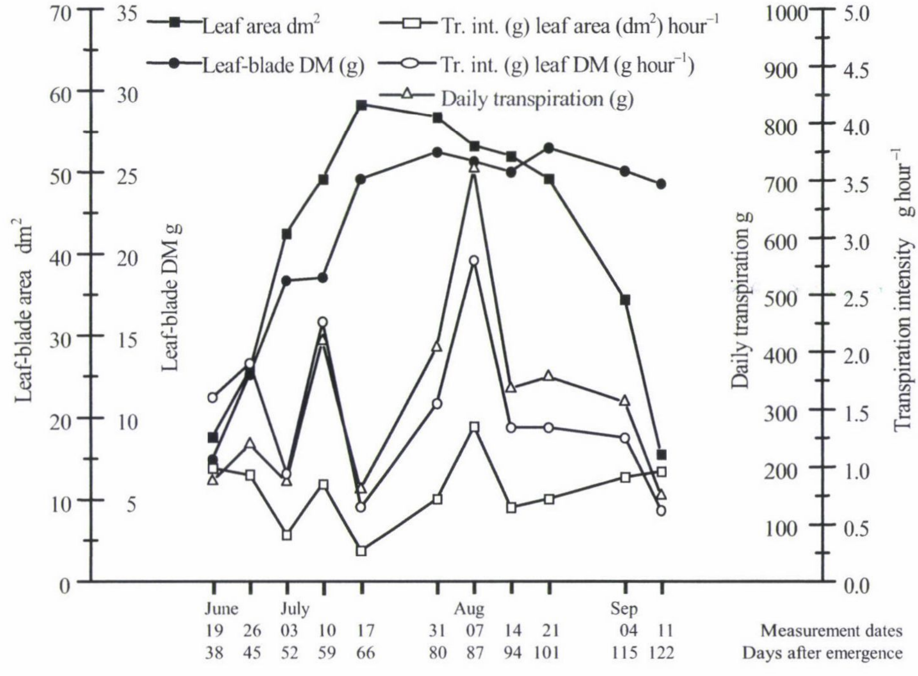

Fig. 3. Dynamics of maize transpiration in the first year

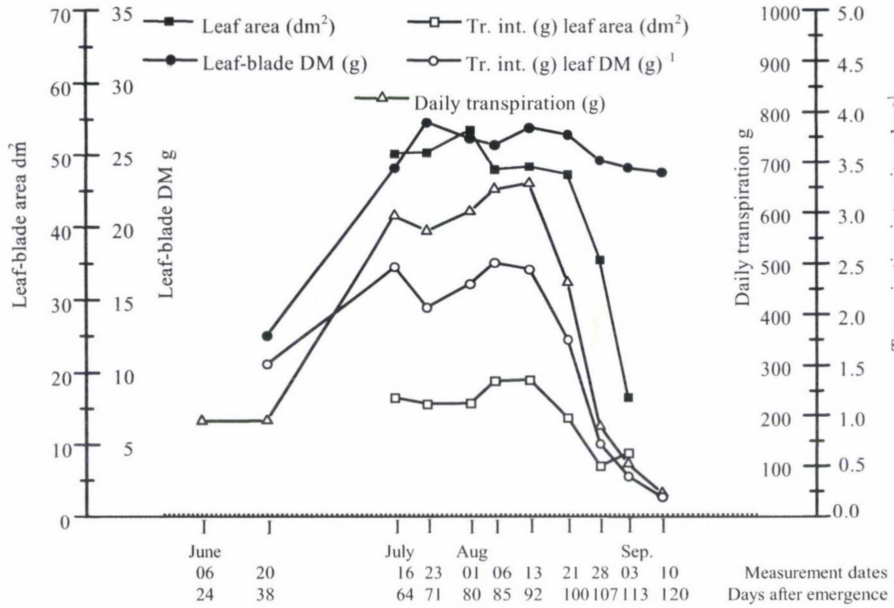

Fig. 4. Dynamics of maize transpiration in the second year

Phosphorus uptake

Plants not only absorb phosphorus, but also release it. Hevesy (1945) reported that for every six P atoms absorbed by the roots, one was lost, i.e. more P atoms are taken up by roots than can be found in the plant.

In the present experiment the total phosphorus uptake in the first year increased in all plant parts up to July 31st, i.e. to the 80th day after emergence, after which, with the exception of the grain, it decreased to the final harvest (Fig. 5). Hanway (1963) also indicated that, after reaching maximum values, P declined in all plant parts except the grain. At the onset of P depletion the leaf-blades and stalk contained most P (as %) in both the first and second years (Table 3). At the onset of DM depletion the P concentration was much lower in the leaf-blades and stalk in both experimental years. The great drop in concentration was due to P translocation to the grain. At the start of dry matter depletion the grain already contained 73.38 and 77.50 % of total P in the plant. At the end of the growth season most P was stored in the grain (85.20%), a substantial part of it as Ca and Mg salts of inositol hexaphosphoric acid (Pethő, 1993). According to Debreczeni (1985) the maize grain contains about 80% of the phosphorus in the whole plant. The second largest proportion of phosphorus was present in the leaf-blades (5.34%), while the least was found in the leaf-sheaths

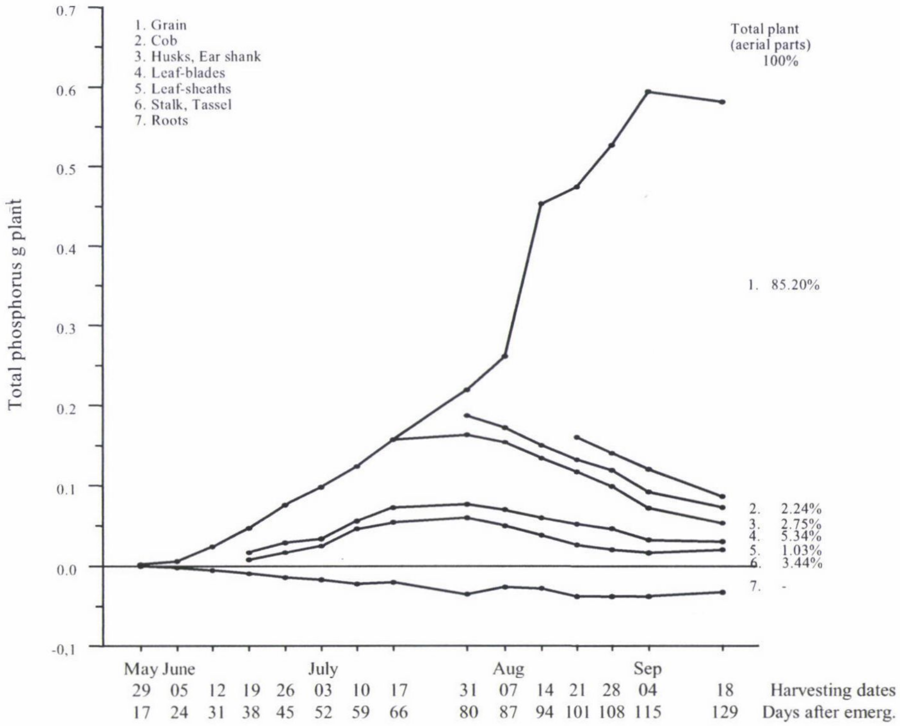

Fig. 5. Total phosphorus uptake and translocation in maize (First year)

(1.03%). A similar phosphorus uptake was observed in the second year (Fig. 6). The total phosphorus content of the plant increased to the 80th day after emergence and following this peak, again with the exception of the grain, it decreased to the final harvest. The grain, as in the previous year, stored 84.51% of the phosphorus in the aerial organs of maize, while the second largest proportion of phosphorus was again stored in the leaf-blades (5.92%). The least phosphorus was found in the tassel (0.43%). If the phosphorus content of the tassel was ignored, the lowest phosphorus content was found, in the leaf-sheats (1.65%, as in the previous year.

Table 3

Total phosphorus distribution (%) in plant parts with the onset of P and DM depletion in the shoot

Year	Days after emergence	Plant parts					
		Stalk	Leaf-sheaths	Leaf-blades	Husks	Cob	Grain
First	80	27.40	7.76	31.05	10.96		
	108	3.80	4.30	10.08	3.40	5.05	73.38
Second	80	31.48	6.14	24.55	9.39		
	107	5.22	2.50	9.32	2.50	2.95	77.50

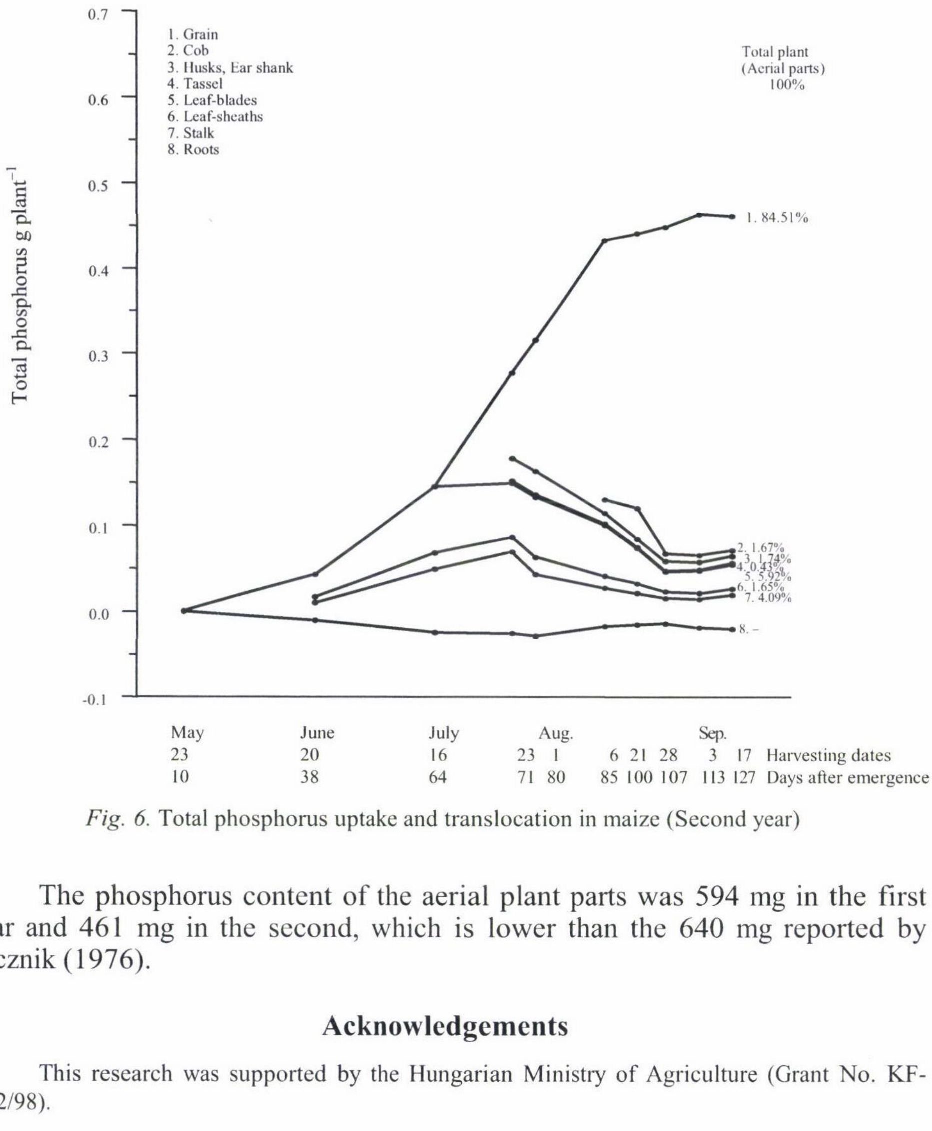

Fig. 6. Total phosphorus uptake and translocation in maize (Second year)

The phosphorus content of the aerial plant parts was 594 mg in the first year and 461 mg in the second, which is lower than the 640 mg reported by Pecznik (1976).

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ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SEED IN PEPPER (*Capsicum annuum* L.) VARIETIES

B. KRSTIĆ[1], L. J. MERKULOV[1], Đ. GVOZDENOVIĆ[2] and S. PAJEVIĆ[1]

[1] INSTITUTE OF BIOLOGY, FACULTY OF SCIENCES, NOVI SAD, YUGOSLAVIA
[2] INSTITUTE OF FIELD AND VEGETABLE CROPS, NOVI SAD, YUGOSLAVIA

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A study was conducted on the seed of eight commercial pepper varieties developed at the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia. The analysis of anatomical parameters observed on dissected seeds indicated the presence of significant quantitative differences between the varieties. Significant differences also existed in the dynamics of germination. The analysed seeds did not differ in the contents of nitrogen, phosphorus and sodium, but variability was recorded for the potassium and calcium contents. The variety Atina had the highest contents of macroelements and total ash.

The oil content in the seed ranged from 10.78% to 21.00% (in Vranjska and Matica, respectively). The quantities of fatty acids varied from one variety to the other, but there were no qualitative differences. Pepper seeds had high average contents of unsaturated fatty acids, especially linoleic and oleic (61.00% and 12.8%, respectively).

Key words: pepper, seed, anatomy, oil content, chemical composition

Introduction

Pepper (*Capsicum annuum* L.) is one of the five domesticated species of the genus *Capsicum*. It is a popular food item on account of its high nutritional and biological values. It is either consumed fresh, when the fruit reaches the stage of physiological maturity, or is processed into a seasoning (Stevanović and Miladinović, 1989; Bosland, 1994). The high nutritional value of the pepper fruit is due to the high contents of carbohydrates, oil, malic acid and citric acids, some B vitamins and vitamin C. The market value of pepper is essentially determined by the fruit colour. A high capacity for the accumulation of colouring matter (capsanthin, capsorubin, lutein, β-carotene, cryptoxanthin, zeaxanthin, anthocyanin) is associated with high quality (Minguez-Mosquera and Perez-Galvez, 1998). Capsaicinoids (capsaicin, dihydrocapsaicin) are alkaloid components that confer a distinctive taste to pepper fruits and seeds. Capsaicin accumulates in the fruit placenta and interlocular partitions, from where it is transported into the seeds; the capsaicin level in the pericarp is low (Marković and Vračar, 1998).

Pepper is basically a self-pollinating plant, although a certain percentage of open pollination (insects) occurs. This is important for seed production because the seed quality determines the yield stability and volume (Bosland, 1996). Seed production is an important facet in the economy of overall pepper production. To ensure seed quality, it is necessary to provide the favourable

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growing conditions needed to produce a maximum number of botanically mature fruits. In pepper, seed quality is a variety characteristic, but is at the same time dependent on the biological and agroecological conditions during cultivation (Gvozdenović et al., 1995).

Pepper seeds are flat, thin, kidney-shaped, smooth and pale yellow, with the exception of *Capsicum pubescens*, which has black seeds. The seeds are mostly attached to the central placenta, but some are found attached to lateral placentas. The seed diameter is 3–5 mm and the seed thickness 0.5–1 mm. The 1000-seed mass ranges from 5 to 7 g. A single fruit may contain 70 to 600 seeds (Marković and Vračar, 1998). Seed quality, i.e. the chemical composition of the seed, is an important factor in the production of paprika powder, a popular seasoning, because the seeds are dried and technologically processed together with the pericarp (Minguez-Mosquera et al., 1993).

Considering the importance of seed in the production of quality peppers, the aim of the present research was to determine the anatomical characteristics of the seed of different pepper varieties, to determine the chemical composition of pepper seeds and to conduct qualitative and quantitative analyses on the oil in pepper seeds.

Materials and methods

The study was carried out on the seeds of eight commercial varieties of pepper developed at the Institute of Field and Vegetable Crops in Novi Sad, namely Plamena, Anita, Matica, Novosadjanka, Atina, Una, Vranjska and Krušnica. The varieties were selected for study on the basis of numerous differences in their morphological and physiological characteristics.

The anatomical characteristics of the seed were observed on cross sections made using a freezing microtome. Microscopic measurements included the following parameters: seed and endosperm thickness and width, epidermis and seed coat parenchyma thickness, size of seed coat parenchyma cells and parts of the germ.

The 1000-seed mass was among the physiological characteristics studied. The dynamics of germination was observed over the course of 15 days, in four replications each containing 100 seeds. The seeds were germinated on wet filter paper in a thermostat, at a temperature of 20–25°C.

Before chemical analysis, the seeds were dried at 100°C and ground. Total N concentration in the dry matter was estimated by the standard microkjeldahl method (Nelson and Sommers, 1973). The concentrations of P, K, Ca and Na were determined after dry ashing at 500°C and treatment with HCl. Phosphorus was assayed spectrophotometrically by the ammonium-vanadate-molybdate method (Gericke and Kurmies, 1952). Potassium and sodium were analysed with a flame photometer, and calcium with an atomic absorption spectrophotometer (Sarić et al., 1990).

Oil was extracted from pepper seeds with ethyl ether for six hours using a Soxhlet extractor. After steaming, the oil was kept in flowing inert gas (nitrogen) in the dark for the subsequent determination of oil content and the preparation of methyl esters.

The fatty acid composition of the pepper seed oil was determined by gas chromatography, analysing the methyl esters under the following conditions: a gas chromatograph "Hewlett Packard" seriesII^{plus} with a flame ionization detector (FID) and a capillary column Supelco SP^{tm}2560; injector temperature 220°C, detector temperature 220°C, column temperature 175°C, carrier gas: helium 0.3 ml/min, detector gases: air (240 ml/min), hydrogen (30 ml/min), nitrogen (60 ml/min). Sample size was 1 µl.

Data analysis was done using the multiple range test (Duncan) at the p=0.05 level of significance.

Results and discussion

Anatomical characteristics of the seed

The seed coat, the endosperm and the rounded germ parts are clearly discernible in the cross-sections of mature seeds (Fig. 1). The outer layer of the seed coat, the epidermis, is characterized by U-shaped thickenings of the radial and inner tangential cell walls. The inner tangential wall of the epidermal cell assumes an undulated surface, while the outer wall is covered with a thicker cuticle. On the margin of the seed, the epidermal cells are much larger than on the two flattened sides. Under the outer epidermis there are 2–3 layers of parenchyma cells, but the layers of cells underneath are completely flattened (Fig. 2). The innermost layer of the seed coat constitutes the small cells of the inner epidermis. They are in direct contact with the endosperm. The endosperm cells have slightly thickened walls and contain oil and aleuron grains. The germ, embedded in the endosperm, is curved and resembles the figure "6". Its radicle is located towards the micropyle, while the growing point or plumule is between the two cotyledons (Somos, 1984).

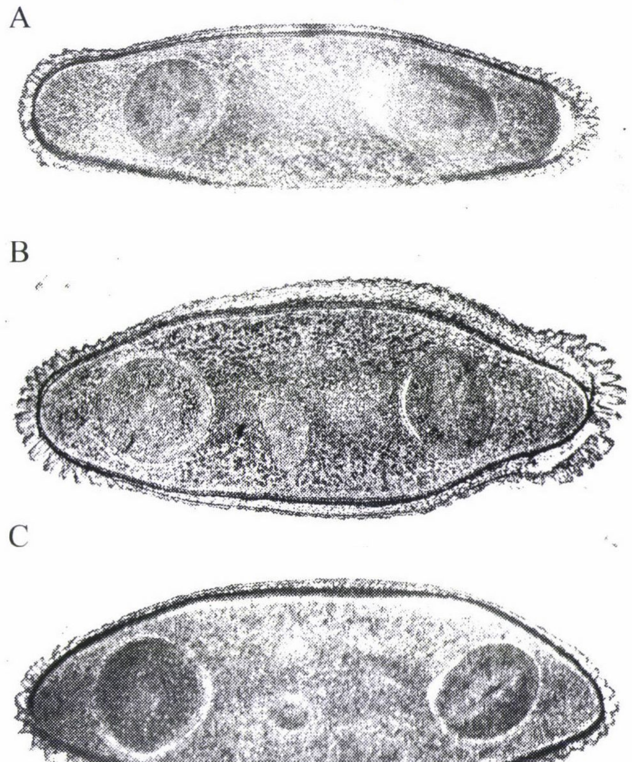

Fig. 1. Seed cross-section of some pepper varieties: A: Plamena; B: Anita; C: Novosadjanka (× 16)

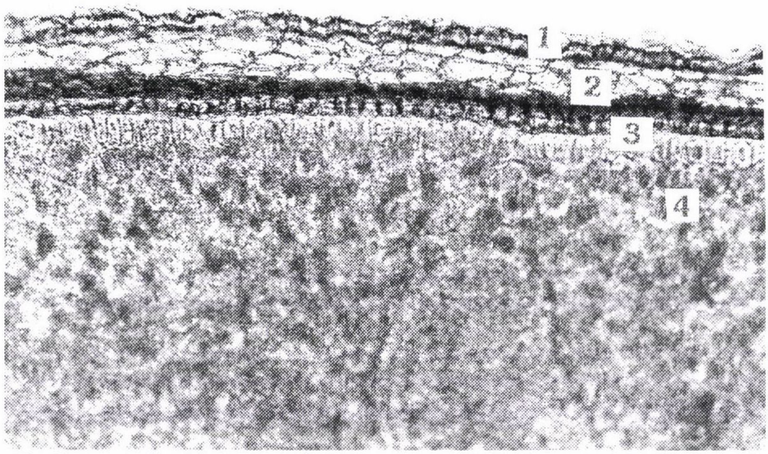

Fig. 2. Cross-section fragment from pepper seed (variety Anita): 1: outer epidermis; 2: parenchyma; 3: inner epidermis; 4: endosperm (× 63)

Considerable quantitative differences were observed between the varieties in the anatomical characteristics of the seed (Table 1). Novosadjanka and Anita had the thickest seeds, and Matica and Plamena the thinnest. The seed width ranged from 3082 μm (Matica) to 3711 μm (Una). The seed widths in Plamena, Novosadjanka and Vranjska were similar. The thickness of the outer epidermis ranged from 20 μm to 30 μm in most of the varieties. The largest difference was found between Novosadjanka and Krušnica, which had values of 15 μm and 30 μm, respectively.

Regarding the thickness of the parenchyma seed coat, the varieties Vranjska and Atina were found to have the highest and lowest values (67 μm and 38 μm, respectively). The values of the other varieties were intermediate and similar. The varieties Una and Novosadjanka had the largest seed coat parenchyma cells, and the variety Atina the smallest.

The endosperm thickness and width correlated with seed size. The highest values of endosperm thickness were found in Novosadjanka and Anita, and the highest values of endosperm width in Una and Vranjska. Matica had the lowest values for both parameters. Una and Anita had the largest values of germ height and width, and Matica the lowest.

Physiological characteristics of the seed

1000-seed mass

Seed mass is an important indicator of endosperm size, i.e. of the amount of nutrients available for the initial growth of the emerging plantlets. Seed size reflects seed quality. This characteristic varies with fruit position on the plant, i.e. from which bloom the fruit comes. A study by Petrov (1964) showed that seed mass in botanically mature pepper fruits is largest in the fruits from the first bloom, then from the second and finally from the third.

Table 1

Nodulation, growth and yield parameters and seed chemical composition of various mungbean varieties (mean of all fertilizer treatments)

Varieties	1	2	3	4	5	6	7	8	9	10	Seed chemical composition (%)		
											Protein	P	K
Kawmy–1	7.00	40.58	82.25	83.12	1.97	47.04	9.15	39.04	38.58	0.2356	23.12	0.54	1.08
VC–4	6.33	41.16	88.37	71.58	2.57	37.62	8.50	42.79	51.66	0.1996	20.99	0.56	1.07
VC–9	8.11	46.29	85.79	73.79	2.63	40.75	9.36	43.83	53.99	0.2146	22.03	0.54	1.06
King	7.94	47.76	90.50	84.66	3.75	45.62	10.29	49.79	62.65	0.2073	21.43	0.58	1.09
$LSD_{5\%}$	NS	0.23	0.25	0.69	0.25	0.25	0.09	0.74	0.52	0.0018	0.30	0.03	0.02
$LSD_{1\%}$	NS	0.43	0.34	0.92	0.34	0.34	0.12	0.99	0.71	0.0024	0.40	0.04	0.03

1: Nodules (No./plant); 2: Days to 50% flowering; 3: Days to 90% maturity; 4: Plant height (cm); 5: Branches (No./plant); 6: Pods (No./plant); 7: Seed yield (g/plant); 8: Biological yield (g/plant); 9: Seed index (1000 seed weight, g); 10: Harvest index; NS = not significant

Table 2

Influence of *Bradyrhizobium* and *Azotobacter vinelandii* on nodulation, growth and yield parameters and seed chemical composition of different mungbean varieties

Varieties / Parameters	1	2	3	4	5	6	7	8	9	10	Seed chemical composition (%)		
											Protein	P	K
Kawmy–1:													
Rh.	6.66	39.75	81.58	82.41	1.95	46.08	9.07	38.08	38.33	0.2398	22.93	0.46	1.07
Rh.+Azot.	7.30	41.41	82.91	83.83	2.00	48.00	9.22	40.00	38.83	0.2313	23.31	0.62	1.09
VC-4:													
Rh.	4.33	40.33	88.00	71.50	2.50	36.25	8.35	42.50	50.70	0.1977	20.41	0.53	1.06
Rh.+Azot.	8.44	42.00	88.75	71.66	2.65	39.00	8.65	43.08	51.66	0.2016	21.58	0.58	1.08
VC-9:													
Rh.	5.66	45.41	85.50	73.25	2.58	40.16	9.26	42.83	53.50	0.2176	21.31	0.53	1.06
Rh.+Azot.	10.55	45.50	86.08	74.33	2.69	41.33	9.46	44.83	54.48	0.2116	22.70	0.56	1.06
King:													
Rh.	6.22	47.25	90.41	84.08	3.51	44.41	10.13	49.08	62.08	0.2073	20.94	0.55	1.08
Rh.+Azot.	9.66	48.16	90.50	84.66	3.75	46.83	10.45	50.50	63.23	0.2074	21.91	0.60	1.09
$LSD_{5\%}$	1.00	0.46	0.34	0.98	0.34	0.36	NS	NS	0.75	0.0025	0.43	0.05	NS
$LSD_{1\%}$	1.34	0.61	0.48	1.30	0.48	0.48	NS	NS	1.00	0.0034	NS	0.06	NS

For legend see Table 1; Rh = *Bradyrhizobium;* Azot.: *Azotobacter vinelandii*

In this study (Table 2), seed mass ranged from 6 to 9 g per 1000 seeds. This was in agreement with data from the literature (Marković and Vračar, 1998). Una and Novosadjanka had the highest seed mass and Atina the lowest.

Dynamics of germination and viability

The tested varieties differed regarding the dynamics of germination and viability (Fig. 3). Some varieties, such as Krušnica and Plamena, reached a germination level of 70% within a few days, while Una germinated slowly. When analysing the dynamics of germination and viability, it is necessary to take into account the conditions under which the seeds were produced and stored, the storage period and the seed size, i.e. the amount of nutrients in the seeds.

Popova (1973) stated that the viability of hybrid seed depended on the seed and germ size as well as on the conditions under which the parents were grown.

A previous study by Gvozdenović et al. (1995) showed that the tested varieties had the highest seed quality at full maturity and during the period preceding this, while seed from technologically mature fruits had low germination energy and viability.

Chemical composition of the seed

Plant growth and development are correlated with the amounts of available nitrogen, while genotypic variability is lower for nitrogen content (up to 15%) than for any other element (Sarić and Krstić, 1983). When analysing the nitrogen content in different parts of the pepper plant, Kaufmann and Vorwerk (1971) found the highest nitrogen content in the leaves (3.6%) and fruits (3.3%), while the contents in the stem and roots were around 2%. The tested pepper varieties had low genotypic variability for N content, which ranged from 3078 mg% in Matica to 3303 mg% in Anita (Table 2).

Table 2

Concentration of macroelements (mg%) and Na (mg%) in pepper seeds

Variety	N	P	K	Ca	Na	1000-seed mass
Plamena	3184a	554a	608c	258bc	11b	7.51b
Anita	3303a	520a	775ab	313a	26a	6.93c
Matica	3078a	527a	750ab	285ab	12b	6.39d
Novosadjanka	3274a	542a	641c	231c	13b	8.85a
Atina	3096a	529a	837a	276b	15b	6.05e
Una	3067a	526a	642c	267b	13b	8.87a
Vranjska	3184a	551a	687bc	276b	14b	6.38d
Krušnica	3216a	541a	600c	276b	11b	6.21de
$LSD_{5\%}$	253	31	93	33	4	0.19

Values with the same letter do not differ significantly at the 0.05 level significance.

The P content was much lower than the N content. P accumulation was highest at the stage of full flowering. Regarding the P distribution in the plant parts, the highest P content was registered in the fruits, and somewhat lower contents in the leaves, stem and roots (Mécs, 1974). There were no statistically significant differences in P content in the seed of the tested varieties. The values ranged from 520 mg% in Anita to 554 mg% in Plamena.

Potassium regulates the activity of a large number of enzymes. This is why the K content is so important for the growth of seedlings. K deficiency in peppers reduces the fruit size and number and causes fruit deformations. It affects the mesocarp and cell wall of the fruits, i.e. seed quality (Terbe, 1977). Genotypic variability for the K content of the seed was higher than for the other elements. The highest K content accumulated in the seeds of Atina (837 mg%). Similar contents were found in Anita and Matica on the one hand and in Plamena, Novosadjanka, Una and Krušnica on the other.

Mix and Marschner (1976) studied the role of calcium in the vital processes taking place in the pepper plant and the effects of external and internal factors on the calcium content in considerable detail. According to Marković (1986), the calcium content depends on the variety and the plant part, ranging from 0.60 % to 3.67 %. The calcium content in the seed was relatively small (about 300 mg%) compared with the contents found in the other plant parts.

The data presented in Table 3 show that the tested varieties did not differ to a great extent in K content. The highest K content was found in the seed of Anita and the lowest in Novosadjanka (313 and 231 mg%, respectively).

According to Sanchez-Conde (1970), the sodium content in peppers may reach 1%. Frederick et al. (1962) reported a content of about 10 mg%. The present data (Table 2) showed that the sodium content in the seed was exceedingly low, ranging from 10 to 25 mg%. The variety Anita had a higher sodium content than the other varieties in the experiment.

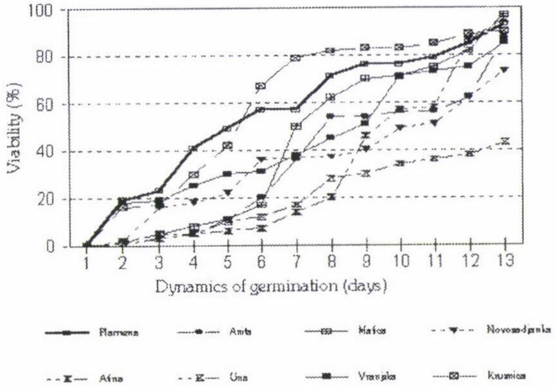

Fig. 3. Dynamics of germination and viability

Oil content and fatty acid composition

The total lipid content in fresh pepper fruits is around 0.4% (Kinsella, 1971). In pepper seeds, however, which make up about 3% of the fruit mass, the lipid content ranges from 10 to 12% (Ćirić et al., 1973). The oil content and the fatty acid composition in the oil determine seed viability and nutritive capacity, characteristics of importance for breeding, especially of spice pepper (Domokos et al., 1993).

The oil content in the seed ranged from 10.78% in Vranjska to 21.00% in Matica (Table 3). Besides Matica, the varieties Una, Krušnica and Novosadjanka had high oil contents in the seed (20.07%, 18.32% and 18.90%, respectively).

The fatty acid composition in pepper seeds was analysed chromatographically. The results obtained are presented in Table 3. Of the 13 fatty acids registered on the chromatograms, the qualitative and quantitative characteristics of six were identified and analysed. The proportion of the latter fatty acids in the total content was significant.

Among the unsaturated fatty acids, linoleic and oleic acids were present in the highest percentages (61.00% and 12.80%, respectively). These two fatty acids made up 74.00% of the total fatty acid content. Palmitic and stearic acids were also present in notable amounts (15.42% and 3.92%, respectively).

A comparison of the results with those obtained by Biacs and Gruiz (after Somos, 1984) showed similar trends, except that Biacs and Gruiz also analysed acids which were present in smaller proportions in the extract. In their study, the dominant acids were linoleic, oleic and palmitic, with 47.30%, 15.30% and 14.10%, respectively.

Ćirić et al. (1973) registered high percentages of linoleic, palmitic and oleic acids in pepper seeds (75.00%,12.60% and 8.79%, respectively).

A comparison of the proportion of fatty acids in the different varieties showed that Plamena and Krušnica had the highest contents of linoleic acid (72.37% and 68.83%, respectively), while Atina had a low content (52.32%). The reverse situation occurred for the content of oleic acid, with Atina in the first place and Plamena in the last.

The content of palmitic acid was high in Vranjska and Atina, and low in Plamena.

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INFLUENCE OF ZINC-ENRICHED ORGANIC MANURES ON THE YIELD, DRY MATTER PRODUCTION AND ZINC UPTAKE OF MAIZE

M. R. LATHA, P. SAVITHRI, R. INDIRANI and S. KAMARAJ

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY,
TAMIL NADU AGRICULTURAL UNIVERSITY,
COIMBATORE, INDIA

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A field experiment was conducted to study the influence of zinc-enriched organic manures on a maize crop. Organic manures, namely farmyard manure, poultry manure, coir pith and biogas slurry enriched with 0, 12.5 and 25.0 kg $ZnSO_4$ ha^{-1} were evaluated for their influence on dry matter production, yield and uptake of zinc in maize. The results revealed that the application of poultry manure was better compared to other sources, resulting in a 26.6% increase in yield. By resorting to the enrichment of poultry manure with zinc, it was possible to save 12.5 kg $ZnSO_4$ ha^{-1}, thereby saving the cost of zinc fertilizer.

Key words: maize, zinc-enriched organic manures, yield, zinc uptake

Introduction

For sustainable agriculture, it is necessary for the soil to supply the essential nutrients that are required for crop growth. The increased removal of micronutrients as a consequence of the adoption of high-yielding varieties and intensive cropping together with a shift towards high analysis NPK fertilizers has resulted in the depletion of micronutrients, among which zinc has gained considerable attention owing to the widespread deficiency noticed in most soils and crops. In India, more than 47% of soils have been reported to be deficient in zinc and the figure for Tamil Nadu is 52% (Anon, 1986). The zinc level required in soils to meet the needs of crops and cropping systems can be met through various sources like inorganic salts, synthetic chelates, organic manures and waste materials. Zinc sulphate is a major source of zinc fertilizer. The use efficiency of applied zinc is low, usually ranging from 5–10% due to its fixation and precipitation in unavailable forms (Mortvedt, 1994). The enrichment of organic manures with inorganic fertilizers leads to the increased use efficiency of applied nutrients. Among the crops maize is the most responsive to zinc after rice (Takkar and Mann, 1975). The application of 5.5 kg ha^{-1} of Zn (25 kg $ZnSO_4$ ha^{-1}) has been reported to alleviate the zinc deficiency in maize (Takkar, 1991). So the present study was undertaken to assess the influence of zinc-enriched organic manures on the yield and uptake of nutrients by maize crops.

Materials and methods

The field experiment was conducted in Tamil Nadu Agricultural University, Coimbatore in Eastern Block Farm during April–August, 1996. The experimental site is located at 11°N latitude and 77°E longitude at an altitude of 427 m above mean sea level. The soil of the experimental site was black, calcareous, belonging to the Inceptisols (Vertic Ustochrepts) and the texture was sandy clay loam. The fertility status of the soil was classified as low in alkaline $KMnO_4$-N (168 kg ha^{-1}), medium in Olsen-P (13.2 kg ha^{-1}), high in NH_4OAc-K (710 kg ha^{-1}) and deficient in DTPA-Zn (1.12 mg kg^{-1}). The test crop (maize var. CO 1) was raised with a spacing of 60 × 20 cm. The experiment was laid out in a factorial randomised block design with fifteen treatments and three replications with a plot size of 5 m × 4 m. The treatment details are given below.

$M_0 Z_0$ - Control	$M_1 Z_0$ - Farmyard manure (FYM) alone
$M_0 Z_1$ - 12.5 kg $ZnSO_4$ ha^{-1} alone	$M_1 Z_1$ - FYM + 12.5 kg $ZnSO_4$ ha^{-1}
$M_0 Z_2$ - 25 kg $ZnSO_4$ ha^{-1} alone	$M_1 Z_2$ - FYM +25 kg $ZnSO_4$ ha^{-1}
$M_2 Z_0$ - Poultry manure (PM) alone	$M_3 Z_0$ - Coir pith (CP) alone
$M_2 Z_1$ - PM + 12.5 kg $ZnSO_4$ ha^{-1}	$M_3 Z_1$ - CP + 12.5 kg $ZnSO_4$ ha^{-1}
$M_2 Z_2$ - PM + 25 kg $ZnSO_4$ ha^{-1}	$M_3 Z_2$ - CP + 25 kg $ZnSO_4$ ha^{-1}

$M_4 Z_0$ - Biogas slurry (BGS) alone
$M_4 Z_1$ - BGS + 12.5 kg $ZnSO_4$ ha^{-1}
$M_4 Z_2$ - BGS + 25 kg $ZnSO_4$ ha^{-1}

The manures required for individual plots of 20 m^2 size were weighed and mixed with the required quantities of $ZnSO_4$ and sufficient moisture. They were then kept for 30 days, maintaining the water level during the period of incubation. At the end of this period, the enriched manures were weighed individually for each plot and applied at a rate of 1 t ha^{-1} as per the treatment schedule before sowing. The normal recommended dose of 135: 62.5: 50 kg N, P_2O_5 and K_2O (Crop Production Guide, 1994) was applied uniformly to all the treatments. One-fourth of the N and the entire P_2O_5 and K_2O were applied basally as urea (46% N), single superphosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively. The remaining nitrogen was top dressed in two splits at 25 and 45 days after sowing. Zinc sulphate (22% Zn) was the source of zinc used in the experiment. Other routine cultural practices were followed as per the recommendations of Tamil Nadu Agricultural University to maintain good crop growth. Five plants were removed at the vegetative and tasselling stages from each plot and the samples were initially air-dried and subsequently oven dried at 60°C to constant weight and the dry matter production was computed and expressed in kg ha^{-1}. The yield of grain and stover for each treatment was recorded at the harvest stage. The plant samples were collected at the vegetative, tasselling and harvest stages, processed and analysed for their zinc contents following the standard procedures of triple acid extraction (Piper, 1966) and atomic absorption spectrophotometry (Jackson, 1973), after which the uptake was computed using the data on DMP, grain and stover yields and their respective zinc contents for the individual treatments.

Results and discussion

The influence of zinc on dry matter production (DMP) during the crop growth period exhibited an encouraging trend (Table 1). The increasing trend might be due to accelerated physiological activity as a result of the increased production of carbonic anhydrase and chlorophyll content induced by zinc application, as stated by Seethambaram and Das (1985). The application of poultry manure and biogas slurry had a profound influence on DMP at various stages due to the inherent higher nutrient contents (Table 2).

Table 1
Influence of zinc-enriched organic manures on dry matter production (kg ha^{-1}) at different stages of crop growth

Treatments	Vegetative stage			Mean	Tasselling stage			Mean
	Z_o	Z_1	Z_2		Z_o	Z_1	Z_2	
No manure	1715	1923	2267	1968	5227	5520	5757	5501
FYM	2110	2362	2547	2340	6280	6452	6661	6464
PM	2292	2584	2830	2569	6361	6560	6767	6563
CP	1961	2234	2484	2226	5956	6256	6521	6244
BGS	2259	2543	2760	2521	6338	6564	6776	6559
Mean	2067	2329	2578	2325	6032	6272	6496	6266

	Zinc	Manure	Stages	M × S
SEd	28	36	28	51
CD (P = 0.05)	57	73	57	104

Table 2
Chemical analysis of organic materials under study

Nutrient	FYM	PM	CP	BGS
N	1.3	1.85	0.68	1.35
P	0.50	0.81	0.10	0.35
K	1.20	1.60	0.78	1.30
Fe	1240	1360	750	1280
Zn	225	290	100	260
Cu	2.50	7.20	3.25	3.10
Mn	75	210	60	80

Major nutrients (%); Micronutrients (mg kg^{-1})

Both grain and stover yield (Table 3) were remarkably high after the incorporation of zinc-enriched organic manures at 1 t ha^{-1}. The yield of maize was increased by 16.0% by 25 kg $ZnSO_4$ ha^{-1} application over the control, and among the manures, poultry manure exhibited superiority over other sources with an increase of 26.6 % over the control. This could be attributed to the enhanced availability of N, P, K, Fe, Cu and Mn besides zinc. Similar results were observed by Takkar and Mann (1975), Prasad et al. (1995) and Thennarasu (1994). Among the manures, poultry manure excelled the others followed by biogas slurry, FYM and coir pith. The efficacy of poultry manure as a source of zinc and as a complexing agent was also reported by Prasad et al. (1984). The difference in yield for the application of poultry manure enriched with 12.5 and 25 kg $ZnSO_4$ ha^{-1} was only marginal for both grain and stover, indicating the possibility of saving 12.5 kg $ZnSO_4$ ha^{-1} when resorting to the enrichment of poultry manure with zinc. The application of zinc-enriched organic manures increased the zinc uptake (Tables 4 and 5) of maize irrespective of the stage of crop growth. These results confirm the findings of Dhillon and Dhillon (1983) and Devarajan et al. (1988). A good deal of variation was observed in the effect of organic sources and levels of zinc on the uptake of zinc, which could be ascribed to the difference in the micronutrient composition and chelating capacity of the manures, as stated by Maskina and Randhawa (1983). Earlier reports by Devarajan et al. (1980) in sorghum and Gupta et al. (1992) in wheat and soybean reiterate the positive effect of zinc-enriched organic manures in increasing the content and uptake of zinc. The mitigating action of organic manures in calcareous soil by avoiding the precipitation of zinc, thereby enhancing its availability and uptake, was reported by Raj and Gupta (1986) and also holds good in the present investigation.

Table 3

Influence of zinc-enriched organic manures on grain and stover yield of maize (kg ha^{-1})

Treatments	Grain yield			Mean	Stover yield			Mean
	Z_0	Z_1	Z_2		Z_0	Z_1	Z_2	
No manure	4675	5197	6055	5309	9604	10493	11477	10525
FYM	5735	6250	6627	6204	11427	12593	13567	12529
PM	6422	6837	6907	6722	12867	13687	13770	13441
CP	5886	6096	6687	6223	11958	12223	13520	12567
BGS	5915	6558	6905	6459	11827	12120	13787	12578
Mean	5727	6188	6636	6183	11537	12223	13224	12328

	Zinc	Manure	Z × M	Zinc	Manure	Z × M
SEd	55	72	124	64	83	139
CD (P=0.05)	113	146	253	131	169	284

Table 4

Influence of zinc-enriched organic manures on the uptake of Zn at vegetative (Veg.) and tasselling (Tas.) stages (g ha^{-1})

Treatments	Z_0			Z_1			Z_2		
	Veg.	Tas.	Mean	Veg.	Tas.	Mean	Veg.	Tas.	Mean
No manure	42.4	116	79.2	46.8	127	86.7	60.5	144	102
FYM	64.5	176	120	76.9	203	140	90.4	232	161
PM	84.9	212	148	99.9	247	174	118	245	182
CP	45.2	130	87.6	52.5	142	97.3	66.7	162	115
BGS	71.1	177	124	87.9	219	153	99.7	241	170
Mean	61.6	162	112	72.8	188	130	87.1	205	146

	Zinc	Manure	Stages	Z × M	M × S
SEd	1.82	2.35	1.82	4.07	4.07
CD (P = 0.05)	3.71	4.80	3.71	8.31	8.31

Table 5
Influence of zinc-enriched organic manures on the uptake of Zn in maize (g ha^{-1}) at harvest

Treatments	Grain			Mean	Stover			Mean
	Z_o	Z_1	Z_2		Z_o	Z_1	Z_2	
No manure	109	132	165	135	191	235	291	239
FYM	156	176	202	178	324	379	439	381
PM	198	220	231	216	422	483	481	462
CP	152	167	184	168	245	275	343	288
BGS	167	200	229	199	333	390	476	400
Mean	156	179	202	179	303	353	406	354

	Zinc	Manure	Z × M	Zinc	Manure	Z × M
SEd	2.4	3.1	5.3	7.0	9.0	15.6
CD (P =0.05)	4.9	6.3	10.9	14.2	18.4	31.8

Conclusions

The yield of maize was increased by the application of 25 kg $ZnSO_4$ ha^{-1} compared to the control, and poultry manure exhibited superiority over the other manure sources. The application of zinc-enriched poultry manure at 12.5 kg $ZnSO_4$ ha^{-1} recorded similar yields to those obtained using 25 kg $ZnSO_4$ ha^{-1}, indicating the possibility of saving 12.5 kg ha^{-1} when resorting to enrichment techniques. Thus, zinc-enriched organic manures can be considered a boon to the farming community in reducing the bill for fertilizers, apart from maintaining soil fertility by improving the physical, chemical and biological properties of the soil.

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GENOTYPIC AND PHENOTYPIC VARIABILITY, HERITABILITY AND PHENOTYPIC CORRELATION FOR YIELD AND YIELD COMPONENTS IN BREAD WHEAT VARIETIES

K. Z. KORKUT, I. BAŞER and O. BILGIN

DEPARTMENT OF FIELD CROPS, FACULTY OF AGRICULTURE, THRACE UNIVERSITY, TEKIRDAG, TURKEY

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This research was conducted to determine the effect of genetic and phenotypic variability on the yield and yield components of some bread wheat varieties over a period of four years (1995–1998). Experiments were established according to a completely randomised block design with three replicates in the Experimental Field of Tekirdağ Agricultural Faculty, Thrace University. In the present research, genotypic and phenotypic variability, heritability and phenotypic correlation coefficients were estimated for plant height, spike length, number of spikelets per spike, number of spikes per square metre, thousand kernel weight, test weight and grain yield per hectare.

The results of data analyses showed that the highest genotypic variability was obtained for per hectare yield, whereas the highest phenotypic variability values were found for plant height, thousand kernel weight and grain yield. For plant height, thousand grain yield and test weight, the broad sense heritability coefficient was found to be the highest, while it was low for spike length, number of spikelets per spike and number of spikes per square metre.

Key words: bread wheat, genotypic variability, phenotypic variability, heritability coefficient, phenotypic correlation, grain yield

Introduction

Wheat ranks as the first crop in the world and in Turkey in terms of production and area sown. According to 1996 statistical data (Anonymous, 1996) 18.5 million tons of wheat were produced on an area of 9.5 million hectares in Turkey. The Thrace Region produced 6.83% of Turkey's production (1.23 million tons) on 532,000 hectares.

In the 1970s, the introduction of the Bezostaya 1 wheat cultivar into the region resulted in an increase in the grain yield. Bezostaya 1 was followed by many cultivar introductions. Numerous cultivars were introduced into the region without making serious tests over locations and years, thus making it difficult to choose the best variety to produce every year.

The main goal in a breeding programme is to develop cultivars having higher adaptability, yield and quality. Genotypic structure and the environmental conditions determine the yield of a cultivar. Apart from the determination of the morphological, physiological and genotypic structure of a plant, yield components and the determination of their heritability coefficients are crucial to success in a plant breeding programme.

Up to date, much research has been conducted on yield components and their heritability in bread wheat. Amaya (1964) found a low heritability coefficient for grain yield and high heritability for thousand kernel weight and test weight, while Gill et al. (1971) reported high heritability for plant height, low heritability for spike length and medium heritability for thousand kernel weight and grain yield. Heritability coefficients of 0.73, 0.40 and 0.24 were observed for number of spikelets per spike, grain yield and thousand kernel weight (Jain and Aulakh, 1971); 0.61 and 0.19 for grain yield and thousand kernel weight (Kaltsikes and Lee, 1971); 65.62/61.73%, 72.61/77.20% and 74.04/34.56% broad sense and narrow sense heritability coefficients for thousand kernel weight, plant height and number of spikelets per plant (Yurtman, 1975); 0.47, 0.31 and 0.11 heritability coefficients for spike length, thousand kernel weight and number of spikelets per spike (Kesici and Benli, 1978); 48.3/36.8% and 66.7/51.0% broad sense and narrow sense heritability coefficients for thousand kernel weight and number of spikelets per spike (Ma, 1988); 0.86, 0.82, 0.67 and 0.66 broad sense heritability coefficients for test weight, plant height, thousand kernel weight and grain yield (Genç and Yağbasanlar, 1989); 90.23/84.25% and 50.27/69.84% broad sense and narrow sense heritability coefficients in F_2 for plant height and spike length (Mosaad et al., 1990); 0.94, 0.84 and 0.82 heritability coefficients for plant height, spike length and number of spikelets per plant (Mladenov, 1993); high heritability coefficients for plant height, spike length, number of grains per spike and number of spikelets per spike and low heritability for thousand kernel weight (Dechev, 1996); 0.30 and 0.45 heritability coefficients for plant height, 0.07 and 0.39 for spike length and 0.07 and 0.49 for thousand kernel weight (Çıtak, 1999). High variability coefficients were found for thousand kernel weight and grain yield (Genç and Yağbasanlar, 1989), for plant height, thousand kernel weight, biological yield and grain yield (Abdel-Moneim, 1996) and for thousand kernel weight, test weight and grain yield (Yağbasanlar et al., 1996).

This study was conducted to obtain information on genotypic and phenotypic variability, heritability coefficients and phenotypic correlation to utilise in future breeding programmes for the Thrace Region.

Materials and methods

Experiments were carried out according to a completely randomised block design with three replicates in the Experimental Field of Tekirdağ Agricultural Faculty, Thrace University during the 1995–1998 growing periods and nine bread wheat varieties were used as experimental material. Each plot measured 6 m^2 and a sowing rate of 500 seeds/m^2 was used. Fertiliser application rates were 50 kg/ha N and P_2O_5 at sowing, 50 kg/ha N at the end of tillering and 40 kg/ha N at 15–20 days before heading. In the experiments, plant height, spike length, number of spikelets per spike, number of spikes per square metre, thousand kernel weight, test weight and grain yield per hectare were examined.

The results obtained were analysed using the MSTAT statistical computer software programme (Anonymous, 1982). In addition, data were calculated according to Falconer (1960), Kempthorne (1957) and Yıldırım et al. (1979) using the following equations:

$$V_p = V_g + \frac{V_{gy}}{\text{year}} + \frac{V_e}{\text{block} \times \text{year}}$$

where V_p = Phenotypic variance, V_g = Genotypic variance, V_{gy} = Genotype × year interaction variance, V_e = Error variance

$$V_g = \frac{\text{MS for genotype} - \text{MS for genotype} \times \text{year interaction}}{\text{year} \times \text{block}}$$

$$V_{gy} = \frac{\text{MS for genotype} \times \text{year interaction} - \text{MS for error}}{\text{block}}$$

MS = Mean square

$$\text{Genotypic variability coefficient (GVC)} = \frac{\sqrt{V_g}}{\bar{x}} \times 100$$

$$\text{Phenotypic variability coefficient (PVC)} = \frac{\sqrt{V_p}}{\bar{x}} \times 100$$

$\bar{x}$: average mean of the characters

$$\text{Broad sense heritability } (h^2) = \frac{V_g}{V_p}$$

Results and discussion

Mean squares from combined analysis for some of the characters studied are given in Table 1. In addition, means, genotypic variance (V_g), phenotypic variance (V_p), genotypic and year variance (V_{gy}), error variance (V_e), genotypic coefficient of variability (GVC), phenotypic coefficient of variability (PVC), broad sense heritability (h^2) and correlation coefficient (r) for nine bread wheat varieties are given in Table 2.

As can be seen in Table 1, the effect of the genotype, year and their interaction on plant height, spike length and grain yield were highly significant, while the genotype and year × genotype interactions were insignificant.

Table 1
Mean squares of certain characters from combined variance analysis in bread wheat varieties (1995–1998)

Characters	Year	Genotype	Year × genotype interaction
Plant height	2521.259***	1089.329**	40.051**
Spike length	13.856**	1.833**	1.520**
Number of spikelets per spike	30.743**	2.844	2.513
Number of spikes per square metre	139.237**	4953.09	3784.000
Thousand kernel weight	26.159	221.166**	10.881**
Test weight	194.034**	28.267**	5.042
Grain yield per hectare	1443442.901*	2916845.833**	1693055.401**

*, ** significant at p = 0.05 and p = 0.01, respectively

Table 2

Means, genotypic variance (V_g), genotypic and year variance (V_{gy}), error variance (V_e), phenotypic variance (V_p), genotypic variability coefficient (GVC,%), phenotypic variability coefficient (PVC,%) and heritability values (h^2) for the bread wheat genotypes studied

Characters	Mean	V_g	V_{gy}	V_e	V_p	GVC	PVC	h^2
Plant height	84.61	87.440	5.150	19.447	90.340	11.05	11.23	0.97
Spike length	8.41	0.026	0.230	0.601	0.134	1.92	4.32	0.19
No. of spikelets per spike	18.70	0.028	0.213	1.663	0.220	0.88	2.51	0.13
No. of spikes per m^2	469.90	97.420	49.390	3586.440	408.640	2.10	4.30	0.24
Thousand kernel weight	38.40	17.520	1.690	4.108	18.280	10.89	11.13	0.96
Test weight	80.96	1.940	0.310	3.812	2.330	1.72	1.89	0.83
Grain yield per hectare	4390.58	101982.54	319914.78	413076.27	216404.25	8.95	11.15	0.64

The genotypic effect was found to be highly significant for both thousand kernel weight and test weight. From these characters, there was a significant year × genotype interaction for thousand kernel weight and a significant year effect for test weight.

The highest genotypic variability coefficients (GVC) obtained were 11.05% for plant height, 10.89% for thousand kernel weight and 7.27% for grain yield. The lowest genotypic variability coefficients were obtained for number of spikelets per spike (0.88%), spike length (1.912%) and test weight (1.72%). Abdel-Moneim (1996), Yağbasanlar et al. (1996) and Genç and Yağbasanlar (1989) reported similar genotypic variability coefficients for these three characters. For plant height, kernel weight and grain yield, the highest phenotypic variability coefficients were found to be 11.23%, 11.13% and 10.59%, respectively. The phenotypic variability coefficients for spike length and number of spikelets per spike were considerably higher than the genotypic variability coefficients; the genotypic variability coefficients were the lowest of all the V_g values.

As for the broad sense heritability, which is expressed by the genotypic variance/phenotypic variance ratio, the highest heritability coefficients were found for plant height (0.97) and thousand kernel weight (0.96). The heritability coefficient for test weight was also high (0.83). It can be concluded from the results that these characters were controlled by genetic factors to a high degree. The estimated heritability coefficients for number of spikelets per spike, spike length and number of spikes per square metre were very low (0.13, 0.19 and 0.24, respectively), indicating that these characters were controlled by environmental factors more than by genotypic factors. The results of these experiments are in line with those of Amaya (1964), Gill et al. (1971), Yurtman (1975), Kesici and Benli (1978), Ma (1988), Genç and Yağbasanlar (1989), Mosaad et al. (1990) and Çıtak (1999), while they differ from those of Jain and Aulakh (1971), Kaltsikes and Lee (1971) Mladenov (1993) and Dechev (1996). The phenotypic correlation coefficients for the examined characters are presented in Table 3.

Table 3

Phenotypic correlation coefficients for the characters studied in nine bread wheat varieties

Characters	Spike length	Number of spikelets per spike	Number of spikes per m^2	Thousand kernel weight	Test weight	Grain yield
Plant height	0.498**	0.242*	–0.159	0.389**	0.158	0.035
Spike length		0.328**	–0.409**	0.238*	0.324**	0.033
Number of spikelets per spike			–0.217	–0.053	–0.076	–0.057
Number of spikes per m^2				–0.071	–0.353**	0.215*
Thousand kernel weight					0.455**	0.456**
Test weight						0.031

*, ** significant at $p = 0.05$ and $p = 0.01$, respectively

The grain yield was found to be positively and significantly correlated with thousand kernel weight and number of spikes per square metre. The phenotypic correlation coefficients of plant height with spike length and number of spikelets per spike; of spike length with number of spikelets per spike, thousand kernel weight and test weight; and of thousand kernel weight with test weight were positively significant, while those of number of spikes per square metre with spike length and test weight were negatively significant. Abdel-Moneim (1996) and Yağbasanlar et al. (1996) reported a similar relationship between grain yield and thousand kernel weight. It can be concluded from these results that thousand kernel weight is an important component significantly affecting grain yield and can safely be used in wheat breeding programmes to enhance the yielding ability.

The analysis of variance showed high genotypic variability coefficients for plant height, thousand kernel weight and grain yield, and high broad sense heritability for plant height, test weight and thousand kernel weight. These results indicated that plant height and thousand kernel weight were strongly affected by the genotypic structure. The broad sense heritability coefficients were low for spike length, number of spikelets per spike and number of spikes per square metre. Therefore, it can be concluded that environmental conditions had a greater effect on spike length, number of spikelets per spike and number of spikes per square metre.

The results obtained in the present study clearly demonstrate that the yielding ability of bread wheat for the Thrace Region could be increased by using thousand kernel weight, plant height and test weight as selection criteria.

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SAPROPHYTIC FUNGI ON TOMATO PHYLLOPLANE: EFFECT OF FUNGICIDES AND LEAF POSITION ON ABUNDANCE, COMPOSITION AND DIVERSITY

C. I. MÓNACO,[1,3] A. I. NICO,[1] H. ALIPPI[1] and I. MITTIDIERI [2]

[1]CENTRO DE INVESTIGACIONES DE FITOPATOLOGÍA (CIDEFI). FACULTAD DE CIENCIAS AGRARIAS Y FORESTALES, UNIVERSIDAD NACIONAL DE LA PLATA, LA PLATA, ARGENTINA
[2]ESTACIÓN EXPERIMENTAL SAN PEDRO, INSTITUTO NACIONAL DE TECNOLOGÍA AGROPECUARIA, ARGENTINA
3 COMISIÓN DE INVESTIGACIONES CIENTÍFICAS DE LA PROVINCIA DE BUENOS AIRES, ARGENTINA

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Fungal isolations were made from leaves of tomato plants cultivated in greenhouses in an area close to La Plata, Argentina. Three different schemes of fungicide application were evaluated: high frequency preventive sprayings (Commercial Greenhouse I), low frequency preventive applications (Commercial Greenhouse II) and no fungicide spraying (Control Greenhouse). Leaves were sampled immediately after second fruit formation from three levels of the foliage: low, medium and high. Plating dilution was used to isolate fungal species. Total c.f.u. number and species composition and diversity were assessed by the plating dilution technique. Fungal populations were most abundant on leaves from lower parts of the foliage in the Control Greenhouse. Diversity varied according to fungicide application frequency and leaf position in the canopy. Higher values were recorded for lower leaves in the Control Greenhouse compared with upper leaves from Commercial Greenhouse II. Likewise position in the canopy influenced the frequency of some species. The implications for natural biological control are discussed.

Key words: biodiversity, biological control, phylloplane, tomato

Introduction

Tomato is one of the most important vegetable crops cultivated in the area of La Plata, Argentina (35° W, 58° S). In commercial greenhouses growers usually develop high input cropping systems in which foliar fungal diseases are controlled by high frequency spraying with preventive fungicides. It has been suggested that the excessive use of fungicides may result in a human health risk both for consumers and for farmers living in the area (De Waard et al., 1993). According to Bollen (1971), fungicide application has two main drawbacks: the introduction of large quantities of toxic substances into ecosystems and the generation of fungicide-resistant strains.

Biological control has been suggested as a suitable method for decreasing the incidence and severity of several foliar diseases (Mónaco et al., 1999). The use of biological control in integrated disease management programmes might result in a decrease in fungicide applications, thus leading to a reduction in pollution.

Regarding biological control in the phylloplane most research has addressed the introduction of foreign antagonistic microorganisms (Blakeman and Fokkema, 1982). Other authors have outlined the importance of indigenous antagonists in suppressing disease and the managing of ecosystem conditions, in order to enhance biological control (Marois and Rouse, 1991). Saprobic fungi inhabiting the phylloplane may exert antagonistic effects on pathogenic fungi, by competition, antibiosis and/or parasitism (Marois and Coleman, 1994). Therefore, microscopic fungi may play an important role not only in pathogenesis but also in the control of foliar diseases (Fokkema, 1993; Andrews and Kenerly, 1978). Restriction or delay in disease onset may be due to the activity of antagonists, which is why the saprophytic fungi of the phylloplane are of interest for natural biological control.

The ability of the indigenous microbial population to prevent pathogenic infections depends mainly on its abundance and diversity. Diversity is important for the effective colonization of different microniches in ecosystems where pathogenesis might take place (Baker, 1991).

Many events that take place on leaf surfaces affect the native saprophytic fungi, quantitatively and qualitatively. A knowledge of the factors that affect the diversity and growth of saprophytic fungi on leaf surfaces would allow us to design a cropping system that would enhance the abundance and diversity of natural antagonists.

The purpose of the work reported here was to evaluate and identify species of fungi inhabiting the phylloplane of tomato from greenhouses with different kinds of management (high input, low input and no pesticide application) and to assess the effect of fungicide application schemes on the abundance and diversity of saprophytic fungi at different canopy levels.

Materials and methods

Sampling of phylloplane fungal flora

Samples were collected from tomato plants cultivated in greenhouses at three different locations in the La Plata green belt. The first sampling was made in a highly technified farm, with five commercial greenhouses devoted to tomato and pepper, managed under a large-scale commercial scheme (Commercial Greenhouse I). The second sampling location was at a smaller farm managed by a family with two greenhouses for tomato cropping (Commercial Greenhouse II). The fungicide application schemes in the two locations are detailed in Figure 1. The third sampling was made in the experimental greenhouse of the Facultad de Ciencias Agrarias y Forestales in La Plata with no pesticide application. Samples were collected in five places equidistantly located along an oblique line with respect to the rows. One plant was selected at each sampling site and three leaves were detached from each: a lower leaf from the third node, a middle leaf from the sixth node and an upper leaf from the ninth node. The combination of fungicide application schemes and leaf canopy positions resulted in nine different treatments.

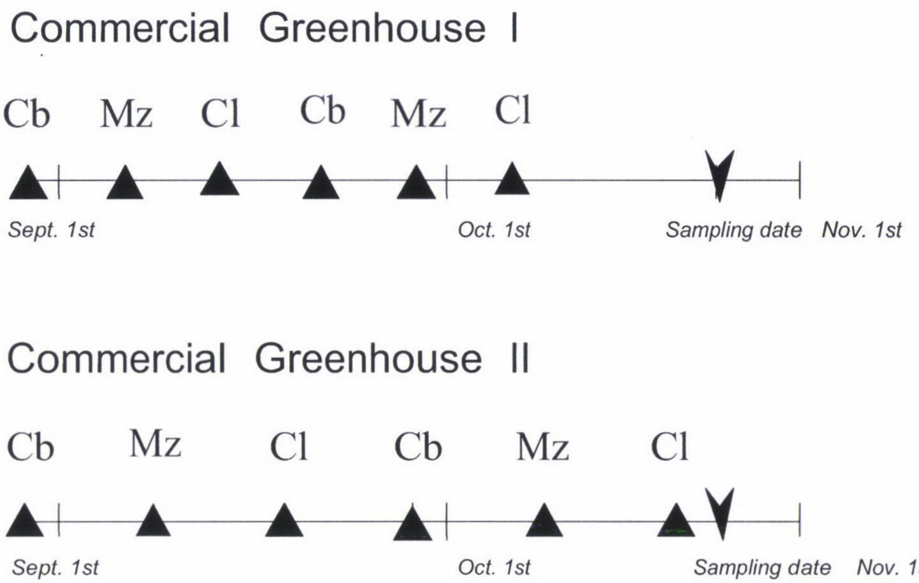

Fig. 1. Fungicide application schemes in the two Commercial Greenhouses. [Cb: Carbendazim (BENCARB®, WP 50%); Mz: Mancozeb (DITHANE® WP 80%); Cl: Chlorotalonil (DACONIL® F 72%)]

Isolation of fungal species

Each leaf sample was dissected into four folioles (subsamples). Then five disks 2 cm^2 in size (16 mm in diameter) were cut from each subsample. These five disks (10 cm^2) were put in 100 ml of sterile distilled water and the mixture was shaken in a magnetic shaker at 120 rev. min^{-1} for 30 min. The suspension was diluted tenfold in sterile distilled water. One ml of this dilution was poured into a Petri dish (9 cm in diameter) to which 10 ml of 2% PDA, oxgall (4 g/l) and 1 ml of streptomycin sulphate solution (250 mg/l) were added. The dishes were incubated at 25±2°C in the dark for 7 days, after which the number of colony forming units (c.f.u.) was assessed and the species were identified.

The experiments were repeated at least twice with at least four replicates. As the results were the same among experiments, statistical analyses were applied to the last experiments.

The data were statistically analysed by ANOVA and the Tukey multiple range test ($P<0.05$).

Diversity

In order to assess the biodiversity of the fungal populations the Shannon and Weaver diversity index (Odum, 1985) was calculated based on the replicates of each treatment. The results were statistically analysed as described above.

Results

The highest number of fungi was found on the lowest leaves of plants grown in greenhouses that had not been treated (Control Greenhouse) (Figure 2). The other treatments resulted in lower levels of fungal populations, except for leaves located in the upper part of the canopy from Commercial Greenhouse I. Leaves from the medium and lower strata of the canopy from the Control Greenhouse showed the highest diversity indexes, while upper leaves from Commercial Greenhouse II had the least diverse fungal populations (Figure 3).

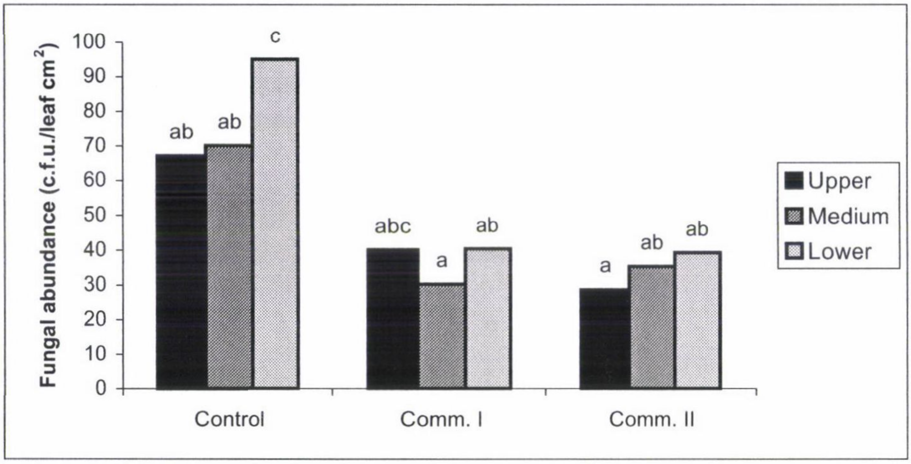

Fig. 2. Abundance of fungal populations on tomato phylloplane. Columns with the same letter do not differ significantly on the basis of Tukey's multiple range test ($P<0.05$)

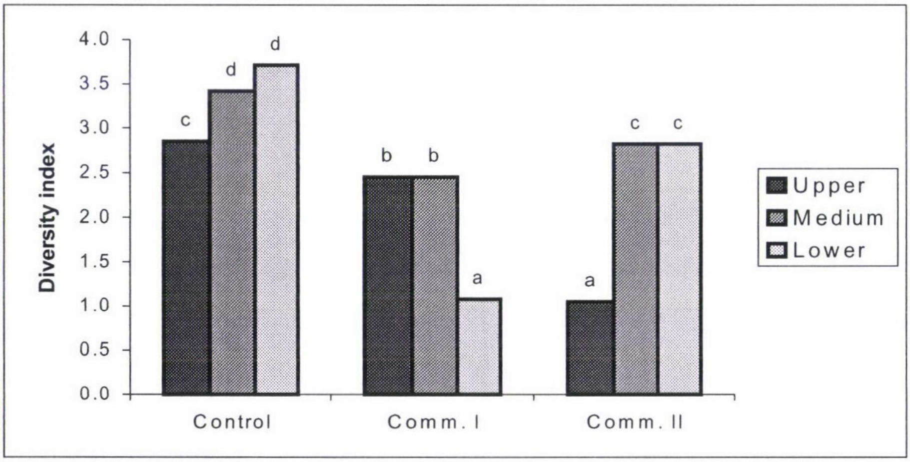

Fig. 3. Diversity of fungal populations (Shannon & Weber index) on tomato phylloplane. Columns with the same letter do not differ significantly on the basis of Tukey's multiple range test ($P<0.05$)

Most of the isolated species were more abundant on tomato leaves from the Control Greenhouse and Commercial Greenhouse II (Table 1). *Cryptococcus luteolus, Rhodotorula* sp., *Arthrinium phaeospermum, Aspergillus* sp (1), *Trichoderma harzianum* (1), *T. polysporum, Penicillium* sp. (1), *Alternaria alternata* and *Cladosporium cladosporioides* had a significantly greater c.f.u. number in the Control Greenhouse than in the other locations. However, the c.f.u. number for some *Penicillium* and *Aspergillus* species was greater in Commercial Greenhouse I.

Leaf position in the canopy influenced the fungal population structure. Some species, such as *Epicoccum nigrum, Chaetomium globosum, Aspergillus* sp. (1), *Trichoderma harzianum* (1), *T. polysporum* and *Penicillium* spp., were more abundant on lower leaves, while *Alternaria alternata, Cryptococcus luteolus, Rhodotorula* sp., *Pleospora herbarum, Fusarium semitectum, F. oxysporum* and *Cladosporium cladosporioides* were present in greater numbers on leaves located in the upper and medium levels.

Table 1
Frequency of saprophytic fungus species on the phylloplane

Species	c.f.u./leaf cm^2 (1)								
	Upper leaves			Medium leaves			Lower leaves		
	CG I	CG II	Control	CG I	CG II	Control	CG I	CG II	Control
Alternaria alternata	0	0	10	0	1.1	2.6	2.6	0	3.6 *
Arthrinium phaeospermum	0	0	0	0	1.6	1.8	0	2.4	3.2 *
Aspergillus niger	0	0	2.6	0	2.3	3.0 *	0	3.6 *	2.7
Aspergillus sp. (1)	3.7	1.1	0	1.4	1.5	1.4	1.2	2.8	2.7
Aspergillus sp. (2)	0	0	0	0	0	0	0	0	3.6 *
Cladosporium cladosporioides	1.5	5.04	5.6	0	3.0	4.7	1.3	4.6	6.4
Cryptococcus luteolus	0	0	10.6	0	2.6	9.5 *	0	1.04	5.4 *
Chaetomium globosum	0	0	0	0	0	3.6	0	1.24	5.6 *
Epicoccum nigrum	0	0	1	0	1.2	1.9	2.4	0	3.6 *
Fusarium oxysporum	0	0	3.7	0	0.98	1.9	0	0	1.16
F. semitectum	0	6.2	0.4	1.4	0	1.9	0	0	2.7 *
Nigrospora sp.	1.4	0	0	0	0.8	1.4	0	1.6	2.7 *
Penicillium sp. (1)	28	13.25	32.6	10.2	12.3	10.4	29	13.7	20.4 *
Penicillium sp. (2)	19	12	4.6	19.1 *	9.8	19.1 *	20.4 *	14.8	17.06
Rhodotorula sp.	0	0	3.7	0	0.8	5.04 *	0	0.5	3.0
Pleospora herbarum	0	0	2.9	0	0	3.6 *	0	0	0
Trichoderma harzianum (1)	0	0	0	0	0	2.1	0	0.8	5.6 *
T. harzianum (2)	0	0	0	0	0	1	0	0	3.1 *
T. polysporum	2.6	0	2.8	5	0	3.6	0	5.8	10.2 *

(1) Values expressed in thousands; CG I: Commercial Greenhouse I, CG II: Commercial Greenhouse II.; *Values significantly greater than others in the same row according to the Tukey test (P<0.05).

Discussion

Both population diversity and fungal abundance were affected by leaf position in the canopy. Fluctuations in temperature and relative humidity decrease towards lower positions in the canopy (Blakeman, 1985). Andrews (1991) suggested the importance of UV light intensity in the regulation of fungal populations in the canopy. This might explain the lower number of fungi recorded on leaves in the upper part of the canopy in both the Control Greenhouse and Commercial Greenhouse II. According to Odum (1985) biodiversity is low in physically controlled ecosystems, i.e. in those subjected to strongly limited physical factors. Leaves located within the canopy might provide a less restricted environment, thus resulting in more diverse communities. Based on the Control Greenhouse and Commercial Greenhouse II, it was concluded that the fungal population was more abundant on lower leaves, while medium and upper leaves had similar fungal populations. According to Andrews (1991) the presence of abundant nutrient sources in lower leaves due to the proximity of the soil might also explain the presence of higher numbers of fungi. On the other hand, Ruinen (1961) asserted that the successional climax of phylloplane microbial populations could not be reached until the leaves reached maturity, and the lower leaves are the most mature ones on the plant. These findings regarding abundance and diversity and their association with position within the canopy, confirm the results obtained for the Control Greenhouse and Commercial Greenhouse II, but not those found for Commercial Greenhouse I, where the upper leaves had more developed fungal populations than any of the other sprayed leaves. The longer time which elapsed (30 days) from the last application in Commercial Greenhouse I might explain this phenomenon. It is important to consider both abundance and diversity when planning natural biological control (Cook and Baker, 1989). So the abundance and diversity of saprophytic fungi achieved in the Control Greenhouse might be more suitable for appropriate disease suppression.

The qualitative composition of the fungal population was also affected by the fungicide application scheme and leaf position in the canopy. Most of the yeasts and fungi found in the upper leaves belong to the naturally-occurring resident microorganisms on aerial plant surfaces listed by Blakeman and Fokkema (1982). The phyllosphere of the lower leaves is inhabited by a number of soil fungi, mainly *Trichoderma* spp. and *Chaetomium globosum*. The ubiquitous genera *Aspergillus* and *Penicillium* have a distribution profile unrelated with leaf position in the canopy. The effect of the fungicide application scheme on the saprophytic fungus composition allows a distinction to be made between fungicide-tolerant species, i.e. those that prevailed and persisted on the leaves from the commercial greenhouses, and susceptible species, which were present only in the Control Greenhouse. The results demonstrated that fungicide application led to the suppression of many potential antagonists of foliar pathogens. Resident yeasts, described by many authors as effective biocontrol agents in the phylloplane (Suzzi et al., 1995; Fokkema and Van der Meulen, 1976) were the most affected by fungicide application. The same was valid for *Trichoderma* spp., an acknowledged biocontrol agent whose population was particularly affected by fungicide applications.

The prevalence of *Aspergillus* spp. and *Penicillium* spp. on leaves from Commercial Greenhouses I and II suggests that fungicide resistance may be the result of the selection pressure performed on both genera (Bollen, 1971). The capacity of some indigenous saprobic phylloplane fungi to develop resistance to fungicides is a desirable characteristic which could be utilised in biocontrol methods (Fokkema and Naaj, 1981; Fokkema, 1993).

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RESPONSE OF A LOCAL AND SOME EXOTIC MUNGBEAN VARIETIES TO BIO- AND MINERAL FERTILIZATION

M. F. EL-KRAMANY[1], A. A. BAHR[1], and A. M. GOMAA[2]

[1]FIELD CROPS RESEARCH DEPT. AND [2]AGRIC-MICROBIOL. DEPT.
NATIONAL RESEARCH CENTRE, DOKKI, CAIRO, EGYPT

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A pot trial was carried out in the greenhouse of the National Research Centre during the summer season of 1999 to investigate the combined impact of *Bradyrhizobium* sp. *(vigna)* and *Azotobacter vinelandii* in the presence of various doses of chemical fertilizers, i.e. 25%, 50% and 100% of the recommended dose of NPK, on nodulation, growth parameters, seed yield and its components, and seed contents of protein, phosphorus and potassium of a local (Kawmy-1) and three exotic (VC-4, VC-9 and King) varieties of mungbean. The results indicated that there were no significant differences between the different varieties for nodule number per plant, while significant variations were obtained between both varieties and biofertilization treatments.

The plants of the Kawmy-1 variety gave the shortest period of growth, the highest number of pods per plant and the highest values of harvest index and seed protein content. The King variety had the longest period of growth and the highest values for number of branches, seed yield, biological yield, seed index and seed phosphorus content. The inclusion of *Azotobacter vinelandii* significantly augmented various tested parameters, with the exception of seed yield, biological yield and potassium content, in comparison with *Bradyrhizobium*. Insignificant differences were found owing to the interaction between varieties, various levels of mineral fertilizers and types of biofertilizers with the exception of harvest index and seed phosphorus content.

Key words: mungbean, biofertilizers, *Bradyrhizobium, Azotobacter vinelandii*, mineral fertilizers

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is a newly introduced pulse crop in several countries, such as Australia, Pakistan, Thailand and Egypt (Ashour et al., 1994). This crop is well known as a summer symbiotic N_2-fixing pulse with a short vegetation period (70–90 days) and high nutritive value.

The seeds contain 22–28% protein, 62–65% carbohydrates, 0.1–1.5% fat, 3.5–4.5% fibre and 4.5–5.5% ash (Lawn and Ahn, 1985). At the present time, the biofertilization of various agricultural crops is of great importance to avoid the heavy application of agrochemicals that cause environmental hazards for plants, animals and human beings. Nowadays, little information is available regarding the response of mungbean to the associative effect of both symbiotic and non-symbiotic biofertilizers.

The present work was planned to study the dual effect of *Bradyrhizobium* sp. *vigna* and *Azotobacter vinelandii* in the presence of reduced doses of chemical fertilizers on a local and three exotic mungbean varieties with emphasis on nodulation, growth, yield and seed chemical composition. In addition, the effect of the different treatments on the maturity stages of the different varieties was compared.

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Materials and methods

A pot experiment was carried out during the summer season of 1999 in the greenhouse of the Field Crops Research Department at the National Research Centre. To study the effect of bio- and mineral fertilization on nodulation growth, seed yield and seed chemical composition of mungbean varieties the experiment included 24 treatments, which were combinations of four varieties (Kawmy-1, VC-4, VC-9 and King), two biofertilizers *(Bradyrhizobium* sp. (*vigna*) and *Azotobacter vinelandii*) and three chemical fertilizers (25, 50 and 100% of the recommended NPK dose: 10 kg N as ammonium sulphate, 30 kg P as single superphosphate: 15.5% P_2O_5, and 20 kg K as potassium sulphate: 48% K_2O). A completely randomized design was used in 5 replicates, giving a total of 120 pots.

Earthenware pots with a diameter of 25 cm were filled with dry clay soil having the following characteristics: pH 8.6, organic matter 2.1%, Ca CO_3 2.6%, total N 0.09%, total P 26 ppm and total K 21 ppm. Each pot was filled with 7 kg dry soil, after which 5 seeds of the respective mungbean variety were sown, namely Kawmy-1 (a registered local variety), VC-4 and VC-9, varieties imported from Taiwan (Asian Vegetable Research for Development Centre, AVRDC) and King (a variety imported from Australia).

Twenty days later, the emerged seedlings were thinned and three plants/pot were left to grow. Irrigation was carried out when needed with equal amounts of water. Superphosphate was added before planting, while the ammonium sulphate and potassium sulphate were divided into two equal doses, the first dose being applied after thinning and the second dose 14 days after the first. Each treatment was replicated five times and each pot represented one replicate.

Biofertilization and method of inoculation

A local isolate of *Bradyrhizobium* sp. *(vigna)* previously identified (Gomaa and El-Kohly, 1999) was grown on YEM (Yeast Extract Mannitol) broth medium (Somasegaran and Hoben, 1985) for 7 days at 28°C. The resultant culture contained 7.3×10^7 cells/ml. In addition, a local isolate of *Azotobacter vinelandii* was isolated from the rhizosphere region of a tomato plant grown in the North Sinai governorate. The isolate was subjected to a detailed identification study according to Bergey's Manual (1998) and was found to have the following characteristics: oval cells in pairs, active motile with peritrich flagella, producing fluorescent green water-soluble pigment in modified Ashby's liquid medium (Abd El-Malik and Ishac, 1968), negative to Gram-strain, capsulated, encysted, positive to catalase test and capable of utilizing mannitol and rhamnose as sole carbon sources but not starch. The strain was grown on modified Ashby's medium (Abd El-Malik and Ishac, 1968) for 21 days at 28°C to obtain a bacterial culture of 6.2×10^6 cells/ml. The cultures were used at a rate of 25 ml/pot.

For seed inoculation, the liquid culture was added separately after sowing directly on to the seeds, so that the seeds in each pot received 5 ml/seed from each culture, according to the respective treatments. The seeds were covered with a thin layer of soil and then irrigated immediately.

Sampling and measured parameters

The nodulation of different mungbean varieties was recorded 45 days after sowing (June 14, 1999). One replicate, containing 3 plants, from each treatment was sampled, while the other four replicates were left to the maturity stage.

Records were made of the days to 50% flowering, days to 90% maturity (90% of pods that had lost chlorophyll pigment), plant height, number of branches/plant, pod number/plant and biological yield/plant. To determine the seed yield/plant, the clean seeds were air-dried till they reached constant weight (12% moisture content) and then weighed to determine 1000-seed weight as well. Harvest index was calculated as seed yield divided by biological yield.

The chemical composition of the yielded seeds, i.e. percentages of protein, phosphorus and potassium, was determined according to the method described by Chapman and Pratt (1978).

Statistical analysis

The data obtained were subjected to analysis of variance for a completely randomized design according to Snedecor and Cochran (1982) where the means of the different treatments were compared by the least significant difference (L.S.D) test at the 5% and 1% levels of significance.

Results

The differences between various mungbean varieties, i.e. Kawmy-1, VC-4, VC-9 and King, regarding nodulation, growth and yield parameters, and the chemical composition of the seeds are presented in Table 1. No significant differences were recorded in nodule number between the different varieties. The highest number of nodules was found for VC-9 and the lowest was recorded for VC-4. Table 1 also shows that the earliest variety for both 50% flowering and 90% maturity was Kawmy-1, while King required the longest period to reach 50% flowering and 90% maturity. With regard to plant height, VC-4 was the shortest, while King was the tallest, with highly significant differences in comparison with the other varieties. King also significantly surpassed the other varieties regarding number of branches per plant, while Kawmy-1 produced the least number of branches.

In the case of yield parameters, Table 1 also indicates that Kawmy-1 yielded the highest number of pods per plant, while VC-4 produced the least number of pods per plant. The differences between the four varieties were statistically highly significant. As to seed yield (g/plant), King produced the highest weight, while the least weight was recorded for VC-4; the differences were highly significant. Highly significant differences were also observed between the four varieties regarding the biological yield (g/plant), with King recording the highest value and Kawmy-1 the lowest. Despite having the highest number of pods, Kawmy-1 produced the lowest 1000 seed weight. The heaviest 1000 seed weight was found for King, and the differences between the varieties were highly significant. With regard to the harvest index, Kawmy-1 significantly surpassed the other varieties, while VC-9 and King came second and third, respectively.

Significant differences were found between the different varieties of mungbean regarding the chemical composition of the seeds (Table 1). Kawmy-1 gave the highest percentage of protein, whereas the lowest percentage was found for VC-4. Although the variation between the different mungbean varieties for phosphorus and potassium contents was not significant, King had the highest values.

Respecting the response of the tested mungbean varieties to biofertilization, i.e. the application of *Bradyrhizobium* sp. *vigna* as a symbiotic biofertilizer and *Azotobacter vinelandii* as a non-symbiotic one, Table 2 indicates that the nodule number/plant of the various mungbean varieties differed significantly. The combined application of *Azotobacter vinelandii* and *Bradyrhizobium* significantly enhanced the nodulation process of various varieties in comparison with *Bradyrhizobium* treatment alone. Although there were significant differences at both the flowering and maturity stages between the varieties, the different biotreatments did not delay the stages of growth compared to the control, but both growth stages were reached a little later when *Bradyrhizobium* and *Azotobacter vinelandii* were applied together compared with *Bradyrhizobium* alone for all the tested varieties. Significant differences were recorded between the different varieties for plant height owing to biofertilization. The shortest mungbean plants were found for VC-4 and the tallest for King. Furthermore, significant differences in plant height were found between the *Bradyrhizobium* treatment and the combined effect of both *Bradyrhizobium* and *Azotobacter vinelandii* for both Kawmy-1 and VC-9, while insignificant differences in plant height were registered the other two varieties. Although there were significant differences between the varieties regarding the number of branches/plant due to biofertilizer application, no significant differences were found between the two biotreatments, i.e. *Bradyrhizobium* and the combination of *Bradyrhizobium* and *Azotobacter vinelandii.* It is worth mentioning that King produced the highest number of branches, while the lowest number was found for Kawmy-1.

As regards the yield and its attributes, a positive correlation was found between the mungbean varieties and the application of biofertilizers (Table 2). Highly significant variations in pod number per plant were recorded both between the test varieties, owing to both biotreatments, and between *Bradyrhizobium* alone and combined with *Azotobacter vinelandii* within the same variety. In general, the highest number of pods was recorded for Kawmy-1.

The combined effect of *Bradyrhizobium* and *Azotobacter vinelandii* resulted in the highest number of pods for Kawmy-1, while the lowest pod number/plant was recorded for VC-4 with *Bradyrhizobium.* With respect to the seed yield and biological yield, King produced the heaviest seed and biological yields, whereas the lowest values were recorded for VC-4 for seed yield and for Kawmy–1 for biological yield, but the differences were not significant. Both seed and harvest index differed significantly, however, due to the interaction between the tested varieties and the biofertilizers. In addition, significant increases were recorded due to the combined application of *Azotobacter vinelandii* and *Bradyrhizobium* for all tested varieties except Kawmy-1, where the difference between the two biotreatments was in-significant. Substantial differences were observed in harvest index, where the greatest significant values were found for Kawmy-1, followed by VC-9 and King. An irregular trend was observed for harvest index due to biotreatment with *Azotobacter vinelandii* and/or *Bradyrhizobium.* For Kawmy-1 and VC-9 the comparison favoured the *Bradyrhizobium* treatment, while in the case of VC-4 the combination of *Bradyrhizobium* and *Azotobacter vinelandii* was superior. For King, an insignificant difference was recorded between the two biotreatments.

Table 1
Anatomical characteristics of seed in pepper varieties (μm)

Variety	Seed thickness	Seed width	Epidermis thickness	Parenchyma thickness	Height of parenchyma cells	Width of parenchyma cells	Endosperm thickness	Endosperm width	Germ height	Germ width
Plamena	1089c	3617ab	23ab	50ab	24.9bc	35.2b	932bc	3235abc	539abc	663b
Anita	1347a	3371bc	23ab	51ab	26.2abc	33.3bc	1122a	3023bcd	628a	727ab
Matica	1087c	3082c	26a	49ab	26.6abc	35.6b	875c	2746d	515bc	530b
Novosadjanka	1347a	3602ab	15b	49ab	30.1ab	36.1ab	1135a	3257abc	604ab	676b
Atina	1122bc	3176c	27a	38b	22.1c	29.0c	960bc	2716d	502c	1021a
Una	1220abc	3711a	20ab	42b	32.2a	41.7a	1021abc	3381a	620a	733ab
Vranjska	1253ab	3622ab	27a	67a	25.8abc	33.9bc	1043ab	3360ab	550abc	661b
Krušnica	1211abc	3344bc	30a	48ab	23.2c	31.1bc	1034abc	2932cd	537abc	709ab
$LSD_{5\%}$	142	2726	9	20.05	6.2	5.7	149	323	89	321

Values with the same letter do not differ significantly at the 0.05 level of significance.

Table 3
Oil content and proportions of fatty acids in the seed of pepper varieties

	Plamena	Anita	Matica	Novosa djanka	Atina	Una	Vranjska	Krušnica	Average
Oil content (%)	15.67	14.67	21.00	18.90	12.24	20.07	10.78	18.32	
Palmitic acid 16:0	11.7	15.2	16.2	17.0	17.2	15.8	17.9	11.9	15.6
Stearic acid 18:0	2.8	4.4	3.1	3.4	5.9	3.0	5.6	3.1	3.9
Oleic acid 18:1	8.7	15.0	11.8	13.3	16.8	10.6	16.1	11.3	12.8
Linoleic acid 18:2	72.4	59.9	64.3	56.9	52.3	63.4	51.9	68.8	61.1
Arachidonic acid 20:0	0.3	0.5	0.4	0.4	0.6	0.3	0.8	0.4	0.5
Linolenic acid 18:3	0.3	–	–	–	–	–	–	0.5	0.4
Other	3.8	5.0	4.2	9.0	7.3	6.8	7.7	4.0	5.9

With regard to the protein content of mungbean seeds of various varieties, it was found that Kawmy-1 significantly exceeded the other varieties. Further, the combination of *Bradyrhizobium* and *Azotobacter vinelandii* resulted in significant increases for all tested varieties in comparison with *Bradyrhizobium*. In the case of phosphorus content, a narrow range of variation was recorded between the different varieties except for Kawmy-1. In general, a significant difference was found between the two biotreatments within the same variety, with the exception of VC-9. On the other hand, no significant differences were recorded for the potassium content of mungbean seeds of different varieties even when the two biotreatments were compared.

Regarding the correlation between varieties, biofertilizers and various levels of chemical fertilizers, insignificant differences were recorded for all the tested parameters, except for harvest index and the phosphorus content of the seeds (Tables 3 and 4). A gradual decrease in the harvest index was observed as the level of chemical fertilizers increased. The harvest index was higher, in most cases, with *Bradyrhizobium* than with the combination of *Bradyrhizobium* and *Azotobacter vinelandii*. On the other hand, the seed phosphorus content increased, in the majority of cases, with an increase in the level of chemical fertilizers. In addition, highly significant differences were recorded due to the combined effect of *Bradyrhizobium* and *Azotobacter vinelandii* in comparison with the effect of *Bradyrhizobium* alone.

Discussion and conclusions

The results obtained indicate that the nodulation parameter did not differ significantly between the tested mungbean varieties. It seems that the interaction between *Rhizobium* sp. and the host plant is plant-type dependent but not variety-type dependent. Nevertheless, *Azotobacter vinelandii* stimulated the nodulation process when applied together with *Bradyrhizobium sp. vigna*. The increase in nodule number/plant due to their combined effect reached 10, 95, 86 and 55%, respectively, for Kawmy-1, VC-4, VC-9 and King over the corresponding biotreatment with *Bradyrhizobium*. These increases could be attributed to the synergistic interaction between the microorganisms and the plant growth promoting substances produced by *Azotobacter vinelandii* (Gonzalez-Lopez et al., 1986) that may enhance the penetration of *Bradyrhizobium* into mungbean roots.

This finding is in accordance with those obtained by El-Bahrawy (1983) and Gomaa and El-Kholy (1999). The shortest period of growth was recorded with the local variety Kawmy-1 (82 days), while the longest period of growth was found for the exotic variety King (91 days). In addition, comparable results for days to 50% flowering and 90% maturity were obtained with both biotreatments for the varieties. Despite the significant difference between the tested varieties in seed yield, these varieties could be successfully cultivated under Egyptian conditions due to the narrow range between them.

Table 3
Growth parameters of mungbean as affected by the correlation between varieties, biofertilizers and various levels of chemical fertilizers

Varieties Treatments	1	2	3	4	5
Kawmy-1					
Rh.+25% NPK	6.00	39.00	81.25	78.50	1.70
Rh.+50% NPK	8.66	40.25	81.50	82.75	2.00
Rh.+100% NPK	5.33	40.00	82.00	86.00	2.15
Rh.+Azot.+25%NPK	9.00	40.75	82.25	81.00	1.90
Rh.+Azot.+50% NPK	8.33	41.50	83.00	84.00	2.00
Rh.+Azot.+100% NPK	4.66	42.00	83.50	84.00	2.10
VC-4					
Rh.+25% NPK	5.33	39.50	87.50	69.50	2.30
Rh.+50% NPK	4.00	40.50	88.00	71.00	2.52
Rh.+100% NPK	3.66	41.00	88.50	74.00	2.70
Rh.+Azot.+25% NPK	9.33	41.50	87.25	70.00	2.45
Rh.+Azot.+50% NPK	9.00	42.00	89.00	71.75	2.70
Rh.+Azot.+100% NPK	7.00	42.50	90.00	73.25	2.80
VC-9					
Rh.+25% NPK	7.66	44.75	85.00	72.00	2.40
Rh.+50% NPK	5.00	45.50	85.25	73.25	2.55
Rh.+100% NPK	4.33	46.00	86.25	74.50	2.80
Rh.+Azot.+25% NPK	13.33	45.00	85.25	73.50	2.42
Rh.+Azot.+50% NPK	9.66	45.25	86.00	74.50	2.80
Rh.+Azot.+100% NPK	8.66	46.25	87.00	75.00	2.85
King					
Rh.+25% NPK	7.66	47.00	90.00	83.00	3.20
Rh.+50% NPK	7.00	47.25	90.50	84.25	3.60
Rh.+100% NPK	4.00	47.50	90.75	85.00	3.75
Rh.+Azot.+25% NPK	10.66	47.50	90.25	84.75	3.70
Rh.+Azot.+50% NPK	10.33	48.00	90.50	85.25	4.00
Rh.+Azot.+100% NPK	8.00	49.00	91.00	85.75	4.25
$LSD_{5\%}$	NS	NS	NS	NS	NS
$LSD_{1\%}$	NS	NS	NS	NS	NS

1: Nodules (No./plant); 2: Days to 50% flowering; 3: Days to 90% maturity; 4: Plant height (cm); 5: Branches (No./Plant); NS: not significant

The application of *Azotobacter vinelandii* together with *Bradyrhizobium* augmented, to some extent, the seed and biological yields of the tested varieties. The same trend was recorded by Gomaa and El-Kholy (1999), who stated that treating mungbean with *Bradyrhizobium*, *Azotobacter chroococcum* and/or *Klebsiella pneumoniae* increased both the seed and biological yields. Furthermore, the seed content of protein, phosphorus and potassium increased due to the associative effect between *Bradyrhizobium* and *Azotobacter vinelandii* (Table 2). These findings are in line with those of Gomaa and El-Kholy (1999).

Except for harvest index and seed P content no significant variations were found in the other parameters due to the interaction between varieties, biofertilizers and various doses of chemical fertilizers. This could be attributed to the low nutritive requirements of mungbean (Abd El-Lateef et al., 1997).

Table 4

Response of mungbean yield and its components to the correlation between varieties, biofertilizers and different levels of chemical fertilizers

Varieties Treatments	1	2	3	4	5	Protein (%)	P (%)	K (%)
Kawmy-1								
Rh.+25% NPK	42.00	8.92	34.00	37.30	0.2628	22.87	0.42	1.07
Rh.+50% NPK	46.25	9.05	39.25	38.70	0.2313	22.92	0.48	1.08
Rh.+100% NPK	50.00	9.25	41.00	39.00	0.2255	23.00	0.49	1.08
Rh.+Azot.+25%NPK	44.00	9.00	36.75	38.00	0.2452	23.20	0.61	1.08
Rh.+Azot.+50% NPK	48.00	9.30	41.25	39.00	0.2255	23.27	0.61	1.08
Rh.+Azot.+100% NPK	52.00	9.37	42.00	39.50	0.2234	23.46	0.63	1.10
VC-4								
Rh.+25% NPK	32.00	8.20	38.00	49.25	0.2157	20.35	0.52	1.06
Rh.+50% NPK	36.75	8.37	43.50	50.35	0.1927	20.27	0.54	1.06
Rh.+100% NPK	40.00	8.50	46.00	52.50	0.1847	20.62	0.54	1.07
Rh.+Azot.+25% NPK	36.00	8.30	38.50	50.00	0.2155	21.15	0.58	1.08
Rh.+Azot.+50% NPK	40.00	8.65	44.00	52.00	0.1965	21.76	0.58	1.08
Rh.+Azot.+100% NPK	43.00	9.00	46.75	53.00	0.1927	21.83	0.58	1.09
VC-9								
Rh.+25% NPK	37.50	9.00	38.00	53.50	0.2368	21.25	0.52	1.05
Rh.+50% NPK	40.00	9.30	43.50	54.25	0.2140	21.30	0.53	1.06
Rh.+100% NPK	43.00	9.50	47.00	55.70	0.2021	21.55	0.53	1.06
Rh.+Azot.+25% NPK	38.00	9.10	40.00	51.00	0.2262	22.25	0.55	1.06
Rh.+Azot.+50% NPK	42.00	9.55	46.00	53.50	0.2075	22.77	0.55	1.07
Rh.+Azot.+100% NPK	44.00	9.75	48.50	56.00	0.2011	23.09	0.57	1.07
King								
Rh.+25% NPK	42.00	9.85	45.00	60.00	0.2194	20.25	0.55	1.07
Rh.+50% NPK	44.75	10.15	51.00	62.75	0.2034	21.10	0.55	1.08
Rh.+100% NPK	46.50	10.40	51.25	63.50	0.1991	21.49	0.56	1.09
Rh.+Azot.+25% NPK	44.00	10.00	45.75	62.25	0.2185	21.00	0.58	1.09
Rh.+Azot.+50% NPK	47.00	10.50	51.75	63.45	0.2028	21.85	0.60	1.09
Rh.+Azot.+100% NPK	49.50	10.85	54.00	64.00	0.2009	22.90	0.62	1.10
$LSD_{5\%}$	NS	NS	NS	NS	00044	NS	0008	NS
$LSD_{1\%}$	NS	NS	NS	NS	00059	–	–	–

1: Pods (No./plant); 2: Seed yield (g/plant); 3: Biological yield (g/plant); 4: Seed index (1000-seed weight g); 5: Harvest index; NS = not significant; Rh = *Bradyrhizobium*; Azot: *Azotobacter*

In conclusion, the exotic varieties VC-4, VC-9 and King could be successfully grown under Egyptian conditions besides the local variety Kawmy-1. Useful results were obtained when a comparison was made between local and exotic mungbean varieties for all tested parameters. King had the highest biological yield, so it could be used as a fodder crop during the summer season. The application of biofertilizers is an urgent necessity to improve the seed yield and its quality, in addition to protecting the environment from the heavy application of chemical fertilizers.

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BIOEFFICACY OF HERBICIDES AND THEIR APPLICATION TECHNIQUES IN COTTON-BASED INTERCROPPING SYSTEMS

A. VELAYUTHAM, A. MOHAMED ALI, V. VEERABADRAN and S. SANBAGAVALLI

DEPARTMENT OF AGRONOMY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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Field experiments were conducted at the Agricultural College and Research Institute (Killikulam), Tamil Nadu Agricultural University, Coimbatore from September 1992 to June 1995 in order to study the bioefficacy of herbicides and their application techniques in cotton-based intercropping systems. The cotton + cowpea intercropping system significantly reduced the total weed density to 42.7 and 53.3 m^{-2} at 20 days after sowing (DAS) and 63.7 and 69.0 m^{-2} at 40 DAS. Metolachlor applied at 1.0 kg ha^{-1} in the form of herbigation (herbicide added to the irrigation water) gave the lowest total weed densities (TWD) of 14.6 and 17.8 m^{-2}, respectively, over the years studied, followed by the same chemical as spray. Weed control efficiency (WCE) was highest (90.7% and 90.5%) with metolachlor at 1.0 kg/ha as herbigation at 20 DAS over the years studied. At 40 DAS metolachlor 1.0 kg/ha as herbigation again gave the highest WCE (88.0%) over the unweeded check. The sole cropping of cotton led to the highest seed cotton yield in both the years (1935 and 1789 kg ha^{-1}). When cowpea was intercropped with cotton, the yield reduction ranged from 39.5 to 125.0 kg ha^{-1}. The cotton + soybean system gave yields of 1899 and 1746 kg ha^{-1} in 1992–93 and 1994–95, whereas the cotton + cowpea system produced seed cotton yields of 1815 and 1659 kg ha^{-1}. Metolachlor at 1.0 kg ha^{-1} applied as herbigation + handweeding at 40 DAS resulted in the best yields (2287 and 2111 kg ha^{-1}), which were significantly superior to those recorded on plots treated with pendimethalin. The next best treatment was metolachlor 1.0 kg ha^{-1} as spray + hand weeding at 40 DAS (2197 and 2033 kg ha^{-1}) and metolachlor 0.75 kg ha^{-1} as herbigation + hand weeding at 40 DAS (2159 and 2006 kg ha^{-1}), which were comparable with each other. Cotton + soybean gave the highest net return (527.85 \$ and 509.20 \$ during the two years of the study) followed by the cotton + cowpea system (492.02 \$ and 462.67 \$). The highest benefit:cost (B:C) ratios of 3.92 and 3.56 for 1992–93 and 1994–95 were obtained for the cotton + soybean system with the pre-emergence application of metolachlor at 1.0 kg ha^{-1} in the form of herbigation + hand weeding.

Key words: cotton weed management, intercropping system, herbicides, herbicide application techniques

Introduction

Cotton is an important commercial crop in India and contributes more than 80% of raw materials to the textile industry. Since it is a wide-spaced, long duration crop, it provides ample scope for raising intercrops to give effective resource utilization with yield advantage and enhanced income from unit area of land. The yield loss due to weeds varies from 21–61% (Sankaran et al., 1993). Intercrops provide efficient coverage of land, resulting in suppressed weed growth. The availability of agricultural labourers for timely weeding may be

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inadequate owing to peak season labour demand. Further, manual weeding is too expensive. Therefore, chemical weed control provides an alternative solution in times of labour scarcity. The weed flora and its competitiveness with crops varies widely due to different cropping systems. The herbicide chosen should be selective for the main and component crops, as well as having little or no residual effect to the succeeding crops. For effective weed control, the uniform application of herbicide is very important. Different herbicide application techniques have been developed to suit various farming conditions. Research over many years has shown herbigation to be simple, economic and reliable (Iruthayaraj, 1981).

The objectives of this study were to evaluate the efficiency of pre-emergence herbicides and their application techniques in cotton-based intercropping systems and to study the residual effect of herbicides on the succeeding sorghum crop.

Materials and methods

Field experiments were conducted at the Agricultural College and Research Institute, Killikulam, Tamil Nadu Agricultural University during the winter season (September–February) for two years in 1992–1993 and 1994–1995. Sorghum was raised as a residual crop during the summer season (February–June) of 1993 and 1995. The soil texture was red sandy loam, classified as typic Rhodostaulf with a pH of 6.6–7.5. The soil was medium in available N (278 kg ha^{-1}), low in phosphorus (26 kg ha^{-1}) and high in potassium (491 kg ha^{-1}).

The experiment was laid out in a split plot design with three replications. The cropping systems formed the main plots, namely cotton sole crop (C_1), cotton + cowpea at 2:2 ratio (C_2) and cotton+soybean at 2:2 ratio (C_3). The following 10 weed management practices were applied in the sub plots: W_1 – Metolachlor at 0.75 kg ha^{-1} as herbigation, W_2 – Metoloachlor at 1.0 kg ha^{-1} as herbigation, W_3 – Metolachlor at 0.75 kg ha^{-1} as spray, W_4 – Metoloachlor at 1.0 kg ha^{-1} as spray, W_5 – Pendimethalin at 0.75 kg ha^{-1} as herbigation, W_6 – Pendimethalin at 1.0 kg ha^{-1} as herbigation, W_7 – Pendimethalin at 0.75 kg ha^{-1} as spray, W_8 – Pendimethalin at 1.0 kg ha^{-1} as spray, W_9 – Hand hoeing and weeding (HW) at 20 and 40 DAS and W_{10} – Unweeded check.

The cotton variety MCU 11, the cowpea variety Co 4, the soybean variety Co 1 and the sorghum variety Co 26 were used. The field was ploughed and levelled, after which beds and channels were formed. Cotton seeds were sown in paired rows of 60×30 cm. In the case of sole cotton, seeds were sown in normal rows at a spacing of 75×30 cm. The intercrops, namely cowpea and soybean, were sown in two rows in between the pairs of cotton rows by adopting a spacing of 30×10 cm after inoculating the seeds with rhizobium. 80:40:40 kg NPK ha^{-1} was applied to the cotton crop as urea, superphosphate and muriate of potash, respectively. Basal dressing of 40:40:40 kg NPK ha^{-1} was applied and 40 kg N ha^{-1} was applied at 45 DAS.

For the herbigation technique, a saline drip bottle (500 ml) was filled with the herbicides without dilution. This was hung downwards at a height of 1.5 m in front of the irrigation channel inlet to the plots in order to drip the herbicide droplets continuously on top of the flowing irrigation water. The quantity of herbicides required per unit area was calibrated considering the quantum of water flow. The herbicides were applied along with life irrigation (second irrigation after the first irrigation on the day of sowing) (3 DAS) in the case of herbigation. For herbicide as spray, treatment was done at 3 DAS followed by life irrigation. In all herbicide-treated plots one hoeing and weeding was given at 40 DAS. Observations on weed vegetation analysis were made using the following methods.

Weed density

A quadrat (0.25 m^2) was placed at four randomly selected places in each plot and the numbers of individual weed species were recorded and expressed as number m^{-2}.

Weed control efficiency (WCE) (Mani et al., 1973)

$$\text{WCE (\%)} = \frac{\text{WPC} - \text{WPT}}{\text{WPC}} \times 100$$

where: WPC – Weed population in control plot; WPT – Weed population in treated plot

Relative density (RD)

$$\text{RD} = \frac{\text{Absolute density of a given species}}{\text{Total absolute density of all species}} \times 100$$

Summed dominance ratio (SDR)

$$\text{SDR} = \frac{\text{Relative density + Relative dry weight}}{2}$$

The seed cotton was harvested in four pickings at weekly intervals. The seed cotton yield obtained from the net plot area at each picking after shade drying was pooled and the total weight was recorded, expressed in kg ha^{-1}. After the removal of the cotton plants, sorghum seeds were sown at a spacing of 45×15 cm.

Results and discussion

Effect of cropping systems and weed management practices on weed vegetation analysis and weed control efficiency (WCE)

The weed flora present in the experimental field included five species of grasses, one species of sedge and ten species of broad-leaved weeds. The grass weeds present were *Echinochloa colona, Cynodon dactylon, Eleusine indica, Dactyloctenium aegytium* and *Panicum* spp. The only sedge species was *Cyperus rotundus*. The broad-leaved weeds present were *Trianthema portulacastrum, Boerhaavia diffusa, Digera arvensis, Portulaca oleracea, Corchorus olitorius, Cleome gynandra, Phyllanthus niruri, Phyllanthus maderaspetensis, Celosia argentea* and *Amaranthus viridis*. The predominance of the above species among the broad-leaved weeds confirms earlier reports in red soil (Alwar Arunachalam et al., 1993). In both the years studied the intercropping system significantly influenced the total weed density at 20 and 40 DAS. The cotton + cowpea system significantly reduced the total weed population (42.7 and 53.3 m^{-2} at 20 DAS and 63.7 and 69.0 m^{-2} at 40 DAS, respectively, during 1992–93 and 1994–95). The cotton + soybean resulted in 45.6 and 53.3 weeds m^{-2} at 20 DAS and 67.7 and 72.1 m^{-2} at 40 DAS in the years studied (Table 1). The highest weed population was recorded in the sole cotton system (66.1, 67.0 m^{-2}, respectively, at 20 and 40 DAS). This may be due to the intercrops growing vigorously during the early stages and covering the soil with their canopy, resulting in reduced weed growth (Balasubramanian et al., 1992). Among the weed management practices at 20 DAS the application of the herbicides metolachlor and pendimethalin had a significant effect in the reduction of total weed density over hand weeding twice and the unweeded check. Metolachlor at 1.0 kg ha^{-1} in the form of herbigation gave the lowest weed numbers of 14.6 and 17.8 m^{-2}, respectively, over the years studied, followed by the same chemical as spray (17.5 and 21.0 m^{-2} during 1992–93 and 1994–95, respectively). At 40 DAS, the unweeded check had the highest weed number m^{-2} (275.3 and 290.5 m^{-2} in the two years). Metolachlor applied as herbigation at 1.0 kg ha^{-1} and growing cotton with intercrops (cowpea and soybean) reduced the density of weeds compared to the unweeded check (Table 2). The effective control of the dominant weeds at the critical period due to metolachlor application at 1.0 kg as herbigation reduced nutrient removal by the weeds and provided a weed-free environment for the better growth of the cotton crop.

Table 1

Total weed density and weed control efficiency as influenced by weed management practices in cotton-based intercropping systems

Treatments	Total weed density (No. m^{-2})				Weed control efficiency (%)			
	1992–1993		1994–1995		1992–1993		1994–1995	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Main plots								
C_1	53.2 (1.577)	78.9 (1.823)	61.9 (1.641)	82.4 (1.841)	68.1	80.7	67.9	80.8
C_2	42.7 (1.477)	63.7 (1.702)	53.3 (1.557)	69.0 (1.744)	79.5	84.4	79.2	84.0
C_3	45.6 (1.519)	67.7 (1.737)	56.8 (1.604)	72.1 (1.778)	77.6	83.5	77.5	83.3
CD	0.005	0.011	0.003	0.025	–	–	–	–
Subplots								
W_1	18.7 (1.314)	37.4 (1.591)	22.5 (1.387)	40.9 (1.663)	88.0	86.4	88.1	85.9
W_2	14.6 (1.221)	32.2 (1.526)	17.8 (1.295)	36.1 (1.577)	90.7	88.4	90.6	87.6
W_3	21.5 (1.368)	40.7 (1.626)	25.9 (1.444)	44.5 (1.665)	86.2	85.3	86.3	84.7
W_4	17.5 (1.289)	35.7 (1.572)	21.0 (1.359)	39.9 (1.619)	88.8	87.1	88.9	86.3
W_5	25.2 (1.434)	53.9 (1.745)	30.2 (1.506)	55.4 (1.758)	83.9	80.4	84.0	80.9
W_6	22.1 (1.379)	45.7 (1.675)	26.7 (1.456)	51.2 (1.725)	85.9	83.4	85.9	82.4
W_7	27.9 (1.470)	57.9 (1.775)	33.3 (1.540)	60.3 (1.793)	82.1	79.0	82.4	79.2
W_8	24.3 (1.418)	51.5 (1.726)	28.8 (1.483)	54.2 (1.749)	84.5	81.3	84.8	81.6
W_9	143.7 (2.159)	70.5 (1.858)	178.6 (2.255)	71.7 (1.861)	8.3	74.4	5.6	75.4
W_{10}	156.1 (2.192)	275.3 (2.443)	188.9 (2.281)	290.5 (2.466)	–	–	–	–
CD	0.010	0.012	0.010	0.032	–	–	–	–
CD (C×W)	0.018	0.018	0.018	0.054	–	–	–	–

Values in parenthesis - Transformed (logx+2) values

Table 2
Relative density of weeds (%) as influenced by weed management practices in cotton-based intercropping systems

Treatments	1992–93						1994–95					
	20 days after sowing			40 days after sowing			20 days after sowing			40 days after sowing		
	G	S	BLW	G	S	BLW	G	S	BLW	G	S	BLW
Cropping system (C)												
C_1	31.8	29.9	38.3	23.2	20.9	55.9	30.7	29.5	39.8	21.9	19.9	58.2
C_2	31.0	29.1	39.9	23.4	19.9	57.7	32.2	28.6	39.2	17.5	19.9	62.6
C_3	33.4	28.7	37.9	23.6	19.2	57.2	31.4	28.1	40.5	20.9	18.3	61.0
Mean	32.1	29.1	38.8	23.4	20.0	56.9	31.4	28.6	39.9	20.0	19.4	60.6
Weed management practices (W)												
W_1	23.4	19.9	56.7	22.1	20.0	58.3	26.4	21.0	52.6	18.0	15.7	66.3
W_2	19.6	17.3	63.1	19.0	16.0	65.1	24.6	19.8	55.5	16.5	13.2	70.3
W_3	23.8	21.1	55.2	24.5	19.5	55.9	25.1	22.3	52.5	20.0	16.6	63.3
W_4	22.2	18.5	59.3	21.4	18.8	59.8	25.4	20.4	54.2	17.7	15.0	67.2
W_5	43.4	39.9	16.7	24.9	21.6	53.5	39.2	36.0	24.8	23.9	22.4	53.7
W_6	44.9	37.7	17.4	23.9	21.3	54.8	39.2	36.3	24.4	21.4	22.6	56.0
W_7	43.4	40.1	16.5	26.5	21.6	51.8	38.0	36.3	25.6	23.3	23.0	53.7
W_8	43.7	39.1	17.2	25.3	21.1	53.5	39.3	36.1	24.6	23.5	22.3	54.2
W_9	28.5	29.5	42.0	23.4	19.3	57.3	28.6	29.3	42.1	22.7	22.2	55.1
W_{10}	28.0	29.1	42.9	23.0	18.1	59.0	29.5	28.5	41.9	22.0	19.4	58.5
Mean	32.1	29.1	38.8	23.4	19.7	56.9	31.5	28.5	40.0	20.9	19.2	59.9

G: Grasses; S: Sedges; BLW: Broad-leaved weeds

Over the years studied, broad-leaved weeds dominated over monocots. There was a relatively low density of sedges compared with grasses and broad-leaved weeds. At 20 DAS, for the cropping systems studied, grasses accounted for about 31.75%, sedges about 28.85% and broad-leaved weeds for 39.4%, whereas the corresponding values for 40 DAS were 21.7%, 19.7% and 58.8%, respectively. With regard to the cropping systems studied, cotton + cowpea and cotton + soybean registered the lowest relative density of *Trianthema portulacastrum* at 20 and 40 DAS over the years studied. Among the broad-leaved weeds, *Trianthema portulacastrum* was the dominant species (35.4–37.0%). The dominance of *Trianthema portulacastrum* was due to its early emergence and development. This was facilitated in the sole cotton crop due to the wider interspace, greater light availability and the slow growth of cotton. When intercrops like cowpea and soybean were raised in the interspace, the growth of *Trianthema portulacastrum* decreased due to the lower availability of space and sunlight and the greater competition from the intercropped pulses. Among the weed management practices studied, metolachlor at 1.0 kg as herbigation resulted in the lowest relative density compared with the other treatments (22.1, 18.6 and 59.3 % at 20 DAS and 17.5, 14.6 and 67.7% at 40 DAS, respectively, for grasses, sedges and broad-leaved weeds). Subramanian et al. (1991) reported that the application of metolachlor at 1.0 kg ha^{-1} was very effective against broad-leaved weeds. The relative density of grasses in plots hand weeded twice did not vary much from the unweeded check plots. The application of metolachlor, irrespective of the levels and methods of application, reduced the relative density of grasses and sedges compared to the unweeded check at both the stages studied. The relative density of broad-leaved weeds was also lower in plots treated with pendimethalin.

The intercropping of cotton with cowpea or soybean led to a lower SDR for grasses (26.48 and 18.97% at 20 and 40 DAS, respectively) and sedges (26.68% and 20.6% at 20 and 40 DAS, respectively). The SDR for broad-leaved weeds was lower in the sole cotton system (45.89 and 57.24 at 20 and 40 DAS) than in the other systems. Among the weed management practices adopted, plots treated with metolachlor had lower values of SDR for grasses and sedges at 20 DAS than at 40 DAS compared to pendimethalin. This indicated that metolachlor application effectively checked the growth of grass and sedge weeds, whereas pendimethalin provided effective control against broad-leaved weeds (Table 3).

Table 3

Summed dominance ratio (SDR) as influenced by weed management practices in cotton-based intercropping systems

Treatments	1992–93						1994–95					
	20 days after sowing			40 days after sowing			20 days after sowing			40 days after sowing		
	G	S	BLW	G	S	BLW	G	S	BLW	G	S	BLW
Cropping system (C)												
C_1	26.72	27.47	45.89	21.44	21.62	56.94	26.86	27.13	45.89	20.98	21.48	57.53
C_2	25.82	26.67	47.38	20.32	20.22	59.96	27.015	26.68	46.28	17.61	20.97	61.41
C_3	27.37	26.55	45.9	21.40	20.55	58.05	26.95	26.82	46.21	20.03	20.7	59.26
Mean	26.64	26.90	46.39	21.05	20.80	58.32	26.99	26.88	46.13	19.54	21.05	59.40
Weed management practices (W)												
W_1	18.09	17.84	63.94	17.77	18.81	63.57	21.12	19.43	59.44	16.23	17.44	66.32
W_2	15.39	15.54	68.85	15.08	14.74	70.22	19.67	18.09	62.17	14.16	14.26	71.57
W_3	19.36	19.71	60.97	19.92	19.69	60.34	20.97	21.09	57.99	18.19	18.86	62.93
W_4	17.32	17.37	65.31	17.21	17.82	64.96	20.46	19.10	60.43	15.69	16.99	67.26
W_5	36.42	35.87	27.70	22.30	23.19	54.50	34.45	33.67	31.87	22.02	23.66	54.31
W_6	37.17	34.00	28.72	20.44	22.61	56.95	34.91	33.26	31.77	19.39	23.63	56.94
W_7	35.7	36.72	27.33	23.22	24.05	52.68	32.98	34.79	32.18	21.93	24.68	53.39
W_8	36.44	35.47	28.09	22.35	22.74	54.84	34.46	33.77	31.77	21.61	23.4	54.95
W_9	25.03	28.25	46.89	23.21	22.01	54.77	25.26	28.05	46.82	28.44	23.58	53.39
W_{10}	25.32	27.71	46.32	29.60	21.41	49.03	26.32	27.44	46.17	20.59	23.58	48.27
Mean	26.62	26.85	46.41	21.11	20.71	58.19	27.06	26.87	46.06	19.82	21.01	58.93

G: Grasses; S: Sedges; BLW: Broad-leaved weeds

WCE was lowest in the cotton sole crop (68.0% and 80.8% at 20 and 40 DAS, respectively), whereas the cotton + cowpea system gave higher WCE values of 79.4% (20 DAS) and 84.2% (40 DAS) over cotton + soybean (77.5% at 20 DAS and 83.2% at 40 DAS). At 20 DAS the WCE for weed management practices varied from 82.0% to 90.7% for the herbicide treatments. The highest WCE values of 90.7 and 90.5% were recorded with metolachlor at 1.0 kg ha^{-1} as herbigation, followed by the same dose as spray (86.3%). All the other weed management practices led to relatively high values of WCE ranging from 82.1 to 88.8% in the first year and 82.4 to 90.6% in the second year. At 40 DAS, metolachlor at 1.0 kg ha^{-1} as herbigation resulted in the highest WCE of 88.0% over the unweeded check, followed by metolachlor 1.0 kg ha^{-1} as spray (86.7%). The handweeding twice plot had a WCE of only 75.0%, whereas the other weed management practices gave WCE values of 74.9% to 86.2%. Muthusankaranarayanan (1994) found that WCE was improved by the use of metolachlor and pendimethalin in chilli.

Effect of weed management practices on seed cotton yield and economic returns

The sole cropping of cotton led to the highest seed cotton yield in both the years (1935 and 1989 kg ha^{-1}). In the intercropping systems, the yield reduction compared to sole cotton ranged from 39.5 to 125.0 kg ha^{-1}. The cotton + soybean system gave yields of 1899 and 1746 kg ha^{-1} and the cotton + cowpea system 1815 and 1659 kg ha^{-1} in the years studied. The higher yield in the cotton + soybean system was due to the better yield attributes of cotton in this system than in cotton + cowpea. The results revealed that the seed cotton yield of cotton was reduced by 1.86–7.27% due to intercropping. Metolachlor at 1.0 kg ha^{-1} applied as herbigation + hand weeding resulted in the highest yields of 2287 and 2111 kg ha^{-1}, which was significantly superior to pendimethalin application and hand weeding twice. This could be due to the greater uniformity of distribution and the thorough coverage of the soil surface with the herbicide, which effectively prevented the emergence of weeds (Babusaravanan, 1992). The next best treatment was metolachlor 1.0 kg/ha as spray + hand weeding (2197 and 2033 kg ha^{-1}) and metolachlor at 0.75 kg/ha as herbigation + handweeding (2159 and 2006 kg ha^{-1}), which were comparable with each other. Pendimethalin at 0.75 kg ha^{-1} as spray+hand weeding produced seed cotton yields of 1800 and 1660 kg ha^{-1}, which was comparable with hand weeding twice. On overall comparison, metolachlor + handweeding was superior to pendimethalin + handweeding and hand weeding twice (Table 4).

Cotton + soybean gave the highest net returns of 527.85 \$ and 509.20 \$ in the two years, followed by the cotton + cowpea system (492.02 \$ and 462.67 \$). Muthusankaranarayanan et al. (1989) reported that though the seed cotton yield was reduced due to intercropping with soybean and cowpea, the net returns and B:C ratio were higher in cotton + soybean due to the additional yield as compared to the cotton + cowpea system. Khistaria et al. (1994) and Patel et al. (1995) reported similar findings. The sole cotton system led to a net return of 445.22 \$ and 442.65 \$ during 1992–93 and 1994–95, respectively. In terms of benefit to cost ratios the cotton + soybean intercropping system registered higher benefit to cost ratios of 3.20 and 2.91 as against 3.07 and 2.73 for cotton + cowpea and 2.89 and 2.67 for cotton sole crop. In both the years, the highest net returns of 649.38 \$ and 641.73 \$ were obtained with metolachlor at 1.0 kg ha^{-1} as herbigation in all the cropping systems. The B:C ratio was also highest for this treatment. Hand weeding twice gave a net return of 449.95 \$ and 427.87 \$. Additional returns of 378.59 \$ and 570.24 \$ ha^{-1} were obtained by adopting various weed management practices compared to the unweeded check. The cotton + soybean intercropping system with the pre-emergence application of metolachlor at 1.0 kg ha^{-1} as herbigation + hand weeding gave the highest net returns (695.40 \$ and 688.20 \$) and B:C ratios (3.92 and 3.56) over the years studied.

Table 4

Yield, net returns and benefit:cost (B:C) ratio as influenced by weed management practices in cotton - based intercropping systems

Treatments		Yield (kg ha^{-1})		Net returns (\$)		B:C ratio	
		1992–93	1994–95	1992–93	1994–95	1992–93	1994–95
Main plots							
	C_1	1935	1789	445.22	442.65	2.89	2.67
	C_2	1815	1659	492.02	462.67	3.07	2.73
	C_3	1899	1746	527.85	509.20	3.20	2.91
CD		19	25	–	–	–	–
Subplots							
	W_1	2159	2006	601.62	595.27	3.59	3.27
	W_2	2287	2111	649.37	641.73	3.79	3.41
	W_3	2078	1912	563.41	553.16	3.41	3.15
	W_4	2197	2033	611.82	603.77	3.57	3.26
	W_5	1865	1719	477.91	460.78	3.00	2.72
	W_6	1989	1826	521.55	504.58	3.10	2.85
	W_7	1800	1660	449.18	431.00	2.88	2.60
	W_8	1907	1758	487.45	471.91	2.96	2.72
	W_9	1784	1641	449.94	427.87	2.93	2.62
	W_{10}	761	652	71.36	25.03	1.32	1.10
CD		64	54	–	–	–	–
CD (C×W)		102	90	–	–	–	–

Residual effect of cropping systems and weed management practices on succeeding sorghum

The cropping systems and weed management practices studied and their interactions did not show any significant effect on the germination percentage of sorghum (Table 5).

In sorghum, *Cynodon dactylon, Echinochloa colona* and *Eleusine indica* were the predominant grass weeds, with the sedge species *Cyperus rotundus*. *Trianthema portulacastrum, Boerhaavia diffusa, Eclipta alba* and *Celosia argentea* were the dominant broad-leaved weeds. Table 1 shows that the cotton + cowpea system had a lower total weed density than the other two systems studied.

The germination of sorghum was not affected by the herbicides applied to the preceding cotton crop. Pendimethalin and metolachlor applied at normal doses did not show any deleterious effect on the germination of the succeeding crop (Muthusankaranarayanan, 1994). The weed management practices adopted in the previous crop of cotton significantly reduced the total weed density in sorghum compared with the unweeded check in both years. During the first year, metolachlor at 1.0 kg ha^{-1} as herbigation led to the lowest total weed density of 30.7 m^{-2} and the hand weeding twice plot gave a total weed density of 99.5 m^{-2}. During the second year the highest reductions in weed density were observed with the above treatments, followed by metolachlor at 1.0 kg ha^{-1} as spray and 0.75 kg ha^{-1} as herbigation. The latter two were on a par with each other.

Neither intercropping or a sole crop of cotton had a positive effect on the grain yield of the succeeding crop. The sorghum yield was lowest (2905 and 2747 kg ha^{-1} during 1992–93 and 1994–95, respectively) after cotton in the unweeded check plots. The adoption of various weed management practices in the preceding cotton crop increased the grain yield of succeeding sorghum by 135 to 235 kg ha^{-1} in the first year and 128 to 228 kg ha^{-1} in the second year. Metolachlor application to cotton at 1.0 kg ha^{-1} as herbigation caused the highest grain yield of sorghum (3140 and 2975 kg ha^{-1}). This was comparable with all the other weed management practices involving pendimethalin application and hand weeding twice adopted in the preceding cotton crop. This might be due to improved plant height, DMP and reduced weed competition in plots where herbicides were applied to cotton. Gill et al. (1987) stated that the application of pendimethalin at 1.0 kg ha^{-1} and metolachlor at 1.0 to 1.25 kg ha^{-1} to sorghum did not show any phytotoxicity and resulted in higher sorghum grain yields (Table 5).

The results of the two-year study indicated that the intercropping system with soybean or cowpea resulted in higher net returns than sole cropping. The net returns and B:C ratio were higher with cotton + soybean followed by cotton + cowpea. Among the weed management practices adopted, metolachlor at 1.0 kg ha^{-1} as herbigation followed by one hand weeding at 40 DAS registered higher net returns and B:C ratio compared to hand weeding twice and the unweeded check. The herbicides applied to cotton did not show any phytotoxicity on succeeding sorghum.

Table 5
Residual effect of weed management practices in cotton-based intercropping system on succeeding sorghum

Treatments		Germination percentage		Weed density (No. m^{-2})		Yield (kg ha^{-1})	
		1992–93	1994–95	1992–93	1994–95	1992–93	1994–95
Main plots							
	C_1	93.5	94.8	1.727	1.769	3030	2884
	C_2	92.4	93.7	1.670	1.726	3081	2922
	C_3	93.3	94.3	1.704	1.740	3070	2912
CD		NS	NS	NS	NS	NS	NS
Subplots							
	W_1	92.3	93.5	1.558	1.610	3114	2950
	W_2	91.2	92.3	1.509	1.569	3140	2975
	W_3	93.0	94.2	1.600	1.649	3104	2938
	W_4	91.8	93.1	1.552	1.602	3126	2961
	W_5	94.3	96.0	1.666	1.707	3068	2893
	W_6	94.0	95.1	1.627	1.669	3086	2923
	W_7	96.3	96.6	1.623	1.747	3048	2888
	W_8	94.6	95.6	1.663	1.703	3074	2905
	W_9	95.2	96.2	2.040	2.019	3040	2875
	W_{10}	98.3	90.3	2.167	2.185	2905	2747
CD		NS	NS	0.078	0.003	120	100
CD (C×W)		NS	NS	NS	NS	NS	NS

NS – Non-significant

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VARIATION OF SUNFLOWER GROWTH, SOIL MOISTURE AND SOIL TEMPERATURE IN RELATION TO PLANTING PATTERNS AT A HIGH LATITUDE SITE

M. LONG[1] and H. EISZNER[2]

[1]INSTITUTE OF PLANT SCIENCES, ETH ZURICH, ZURICH, SWITZERLAND
[2]INSTITUT FÜR ACKER- UND PFLANZENBAU, MARTIN-LUTHER-UNIVERSITÄT, HALLE-WITTENBERG, HALLE(SAALE), GERMANY

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Field experiments were conducted at a high latitude site for sunflower (*Helianthus annuus* L.) production in central Germany (51°24' N, 11°53' E) in 1996, 1997 and 1998. The responses of sunflower development to various planting patterns differed in the duration from emergence to the middle of the linear growth period as calculated via a tangent hyperbolic model $F(t)=(\alpha+\beta)\times\tanh[\delta\times(t-\tau)]$. Final dry matter accumulation showed few differences among the planting patterns: 12 plants m^{-2} at 50 cm row spacing (RS1PD3), 8 plants m^{-2} at 75 cm row spacing (RS2PD2) and 4 plants m^{-2} at 100 cm row spacing (RS3PD1). The actual and simulated values for final dry matter were close to 1200 g m^{-2}. The responses of soil moisture and temperature to planting patterns changed from the upper to the deep soil layers. In a normal year, e.g. 1997, the soil water to 150 cm depth was sufficient for sunflower growth. In a drought year, e.g. 1998, soil water deeper than 150 cm was used by sunflower crops. The soil temperature was mostly lower in RS1PD3 and RS2PD2 than in RS3PD1, particularly in the upper soil, at depths of 5 and 20 cm. The most important factor defining the responses of soil moisture and temperature to planting patterns seems to be the amount of radiation penetrating the ground, which may depend on latitude, wind and row orientation.

Key words: plant density, row spacing, soil temperature, soil moisture, sunflower (*Helianthus annuus* L.)

Introduction

Optimizing crop management may alter canopy establishment and the root system. Canopy structure is decisive in determining the partitioning of radiation between the plant and the soil surface. The growth and distribution of the roots depends to a great extent on the soil environment, while the activities of the roots affect soil moisture and soil temperature. Thus, soil moisture and soil temperature may be modified by changing planting patterns (Tanji and Yaron, 1994). The choice of plant density and row spacing, including row orientation, is the source for changing planting patterns. Many studies have been conducted to investigate the responses of sunflower (*Helianthus annuus* L.) yields to planting patterns, but these gave inconsistent results due to genotype- and site-specificity (reviewed by Long et al., 2001). However, reports on variations in soil temperature and soil moisture due to planting patterns are still scarce.

The average and maximum intensities of irradiance and the angle of incoming radiation vary with changes in the latitude. The response of sunflower growth to planting patterns may depend on the latitude of the experimental site (Long et al., 2001). The objectives of the present study were i) to characterize the responses of sunflower growth, i.e. aboveground dry matter accumulation, to various planting patterns in a marginal area for sunflower production and ii) to assess the seasonal changes in soil moisture and soil temperature due to planting patterns and plant development at a high latitude site.

Materials and methods

Location and field management

Field experiments were conducted at Bad Lauchstädt (51°24' N, 11°53' E, 113 masl) in central Germany on a loamy Haplic chernozem in 1996, 1997 and 1998. This site is a marginal area for sunflower production due to the high latitude. The trials were established on two sites. On site 1, east to west row orientation was used and in site 2, north to south row orientation was used. Except for the difference in row orientation, the field management was completely identical at the two sites. The short-season sunflower cultivar Eurosol was sown on 22 April 1996, 8 April 1997 and 14 April 1998. Nitrogen fertilizer was applied at the recommended rates before sowing. Weeds were controlled by herbicides; no other pesticides were used.

Weather conditions

Precipitation from April to September totalled 283, 368 and 370 mm in 1996, 1997 and 1998, respectively. The long-term (1896–1995) mean for this period was 308 mm. There were periods of drought in June 1996 and May 1998, the latter being particularly severe. The average air temperature from April to September was 13.5, 14.9, 15.6 and 14.4°C for 1996, 1997, 1998 and the long-term mean, respectively. Thus, in 1996, the temperature during the growing season was below the long-term average, while in 1998 it was above the long-term average. Precipitation was low while the temperature was very high in May and June 1998. Nevertheless, precipitation in July was far above the long-term mean in all three years, indicating that the water supply was relatively high during the phase of rapid growth.

Experimental design

The plots (9 × 6 m) were set up in a split-plot design with four replications. The row spacing (50, 75 and 100 cm; referred to as RS1, RS2 and RS3) was the main plot and the plant density (4, 8 and 12 plants m^{-2}; as PD1, PD2 and PD3) the subplot. Additional plots of the same size but located on an adjacent area were used for the stationary measurement of soil moisture and soil temperature. These parameters were assessed in the following treatments: i) 12 plants m^{-2} at 50 cm row spacing (RS1PD3), ii) 8 plants m^{-2} at 75 cm row spacing (RS2PD2) and iii) 4 plants m^{-2} at 100 cm row spacing (RS3PD1).

Parameter measurement and calculation

The phenology of sunflower was recorded weekly. Days after emergence (DAE) and weeks after emergence were also monitored. Aboveground dry matter accumulation was determined at six dates, i.e. 3, 5, 7, 9 and 12 weeks after emergence, and at physiological maturity.

Soil moisture was measured hourly in the central row of the additional plots in two replications using the TRASE SYSTEM 1 (MODEL 6050 X1, Soilmoisture Equipment Co., CA, USA). The measurements were conducted from 29 (1996), 16 (1997) and 19 DAE (1998) to physiological maturity at soil depths of 20, 50, 100 and 150 cm. Similarly, soil temperature was recorded hourly in the central row of the additional plots in one replication with a PT100 with 4-wire resistance (Platinum Resistance Thermometer with a resistance of 100Ω at 0°C, Umwelt-Geräte Technik GmbH, Müncheberg, Germany). The measurements started at 19, 15 and 19 DAE in 1996, 1997 and 1998, respectively, and ended at physiological maturity. In the first two years, the measurements were conducted at soil depths of 20, 50, 100 and 150 cm; in the third year, the soil temperatures at 5, 20, 50 and 100 cm depths were measured. The average values of soil moisture and soil temperature per week were calculated for weeks after emergence and presented in this paper.

Meanwhile, maximum and minimum air temperatures were monitored at an automatic agrometeorological station about 150 m from the experimental sites in all experimental years. Growing degree days (GDD, °C d) with a base temperature of 6°C were calculated as

$$GDD = \sum_{j=1}^{n} (T_a - 6)\Delta t,$$

where T_a is the daily mean of maximum and minimum daily temperatures and Δt is a time step in days. T_a was set to 6 when less than 6°C. The GDD was accumulated from sowing to the end of the vegetative and reproductive stages.

Soil thermal units (STU, °C) with a base temperature of 7.2°C were calculated as

$$STU = \sum_{j=1}^{n} (T_{S20} - 7.2)\Delta t,$$

where T_{S20} is the daily mean of soil temperature at 20 cm depth and Δt is a time step in days. This depth was chosen because the bulk of sunflower roots usually occurs about 20 cm below the soil surface (Long, 1999). When T_{S20} was less than 7.2°C, it was set to 7.2. The STU was calculated from sunflower emergence to the end of the vegetative and reproductive stages.

Data processing

Using the program CADEMO (Computer Aided Design of Experiments and Modelling; Rasch and Jansch, 1989), the optimal growth model $F(t)=(\alpha+\beta)\times\tanh[\delta\times(t-\tau)]$ was chosen from eight functions (Long, 1999) depending on their residual variances to depict the dynamics of aboveground dry matter accumulation.

Results and discussion

Plant development and dry matter accumulation

The duration of the life cycle is determined by climatic factors, by the relative maturity of the genotype, and, to a lesser extent, by planting patterns and soil conditions. In the present study, the effects of planting patterns on sunflower development were marginal. The life cycle of Eurosol, the cultivar used, lasted from 128 to 130 days in the three years of testing. Flowering began at about 10 to 11 weeks after emergence. The years differed markedly in the growing degree days (GDD), which was lower in 1996 than in either 1997 or 1998. In all three years, the variations in the GDD were smaller during the vegetative stage than during the reproductive stage (Table 1).

Kharwara and Sharma (1997) argued that the relationship between soil temperature and crop phenology was closer than between air temperature and crop phenology. In the current study, differences in the soil thermal units (STU) could be detected between the planting patterns and the differences were especially great in the north to south oriented rows at site 2 (Table 1). Except during the reproductive stage in 1998, when there was heavy plant lodging, the smallest STU value was always found in RS2PD2, while the differences between RS1PD3 and RS2PD2 were less than between RS3PD1 and RS2PD2. The STU values for RS1PD3, averaged over the two sites were 1127, 1217 and 1223°C d in 1996, 1997 and 1998, respectively. The differences in the STU in all three years were smaller than those in the GDD. This may confirm that the STU is better than the GDD for predicting plant development (Kharwara and Sharma, 1997).

Table 1

Sum of soil thermal units (STU, °C d, base temperature 7.2°C) for different planting patterns and growing degree days (GDD, °C d, base temperature 6.0°C) for three experimental years. RS1PD3, RS2PD2 and RS3PD1 = 12 plants m^{-2} at 50 cm row spacing, 8 plants m^{-2} at 75 cm row spacing and 4 plants m^{-2} at 100 cm row spacing, respectively

Period	STU						GDD
	Site 1			Site 2			
	RS1PD3	RS2PD2	RS3PD1	RS1PD3	RS2PD2	RS3PD1	
				1996			
Vegetative stage	510	502	519	513	500	523	564
Reproductive stage	610	606	617	620	607	629	748
Total life cycle	1120	1108	1136	1133	1107	1152	1312
				1997			
Vegetative stage	509	493	499	484	502	516	697
Reproductive stage	724	680	706	716	702	711	937
Total life cycle	1233	1173	1205	1200	1204	1227	1634
				1998			
Vegetative stage	562	581	592	544	592	589	639
Reproductive stage	683	684	689	656	677	680	917
Total life cycle	1245	1265	1281	1200	1269	1269	1556

The responses of dry matter accumulation to planting patterns were simulated via the tangent hyperbolic model $F(t)=(\alpha+\beta)\times\tanh[\delta\times(t-\tau)]$ (Table 2). In this model, the sum of α and β is a theoretical value which can be treated as the potential dry matter accumulation. τ refers to the time from emergence to the middle of the linear growth period (Warnstorff and Dörfel, 1999). The accumulation of dry matter exhibited a similar pattern, as indicated by the parameters of the growth model, in the various planting patterns and sites (Table 2). In 1997, 58, 58 and 65 days were required to reach the middle of the linear growth period at site 1 for RS1PD3, RS2PD2 and RS3PD1, and 51, 54 and 65 days at site 2, respectively. The corresponding values in 1998 were 58, 61, 67, 57, 59 and 66 days. Similar differences between the planting patterns were found in 1996, when the values were generally high (Table 2). The time to the middle of the linear growth period was shorter in RS1PD3 than in RS3PD1, while the difference between RS1PD3 and RS2PD2 was marginal. This approach reveals that sunflower crops grown in combinations of dense stands with narrow rows, e.g. RS1PD3 and RS2PD2, developed most rapidly. Row orientation had no noticeable effect on plant development (Table 2).

Surprisingly, the time to the key stages, the middle of the linear growth period (τ) and the end of the vegetative stage (t_v), varied among the experimental years. A long vegetative stage might be the result of environmental conditions. For instance, the duration of the vegetative stage was relatively short due to the high air temperature in April and May 1998. However, factors other than soil or air temperature could affect the duration of the linear growth period. In both 1996 and 1998, τ values were bigger than t_v, while the case was reversed in RS2PD2 in 1997 (Table 2). Soil moisture might be attributable to this difference, since precipitation was rare in June 1996 and May 1998.

Table 2

Parameters of the growth model $F(t)=(\alpha+\beta)\times\tanh[\delta\times(t-\tau)]$ as affected by planting patterns in the three years of testing. RS1PD3, RS2PD2 and RS3PD1 = 12 plants m^{-2} at 50 cm row spacing, 8 plants m^{-2} at 75 cm row spacing and 4 plants m^{-2} at 100 cm row spacing, respectively

Location	Trait	α	β	τ	δ	$\alpha+\beta$
				1996		
Site 1	RS1PD3	548	668	67	0.029	1216
	RS2PD2	599	643	67	0.039	1242
	RS3PD1	–	–	–	–	–
Site 2	RS1PD3	606	627	63	0.043	1233
	RS2PD2	568	631	69	0.033	1199
	RS3PD1	559	654	78	0.027	1213
				1997		
Site 1	RS1PD3	527	628	58	0.029	1155
	RS2PD2	522	530	58	0.046	1052
	RS3PD1	522	634	65	0.027	1156
Site 2	RS1PD3	508	465	51	0.041	973
	RS2PD2	501	528	54	0.045	1029
	RS3PD1	539	619	65	0.030	1158
				1998		
Site 1	RS1PD3	644	644	58	0.056	1288
	RS2PD2	581	613	61	0.045	1194
	RS3PD1	595	593	67	0.052	1188
Site 2	RS1PD3	595	623	57	0.046	1218
	RS2PD2	610	630	59	0.046	1240
	RS3PD1	601	638	66	0.041	1239

Nevertheless, there was hardly any difference in final dry matter accumulation in either the field trials or their respective simulations between the planting patterns used. This confirms the conclusions of Sadras and Trápani (1999), who suggested that sunflower crops have great plasticity to resource availability when comparing growth responses to various plant densities under different environments. The threshold of dry matter accumulation of Eurosol in the region where the trials were conducted was close to 1200 g m^{-2} (Table 2).

Time course of soil moisture

The seasonal changes in soil moisture in relation to the planting patterns differed within the vertical soil layers. The water content of the top soil (20 cm depth) changed sensitively with precipitation and plant growth (Fig. 1). In June 1996, precipitation was rare, far less than in the other two years or the long-term mean, so the soil moisture at a depth of 20 cm decreased rapidly to satisfy plant transpiration and soil evaporation. In early July 1996, mid-July 1997 and mid-June 1998, there was abundant precipitation, and the soil moisture at 20 cm formed a clear peak in all three years of testing, after which it decreased up to physiological maturity (Fig. 1). The responses of soil moisture at 20 cm to planting patterns changed slightly with plant development. Soil moisture at 20 cm was, for the most part, higher in RS3PD1 than in the other two treatments, while the differences were far less during flowering (Fig. 1). The time course of soil moisture at depths of 50 and 100 cm showed different characteristics compared with the upper layer (data not shown). Soil moisture at 50 and 100 cm exhibited a decreasing trend all the time except when a storm took place. The effect of planting patterns on soil moisture decreased from the upper to the deeper soil layers.

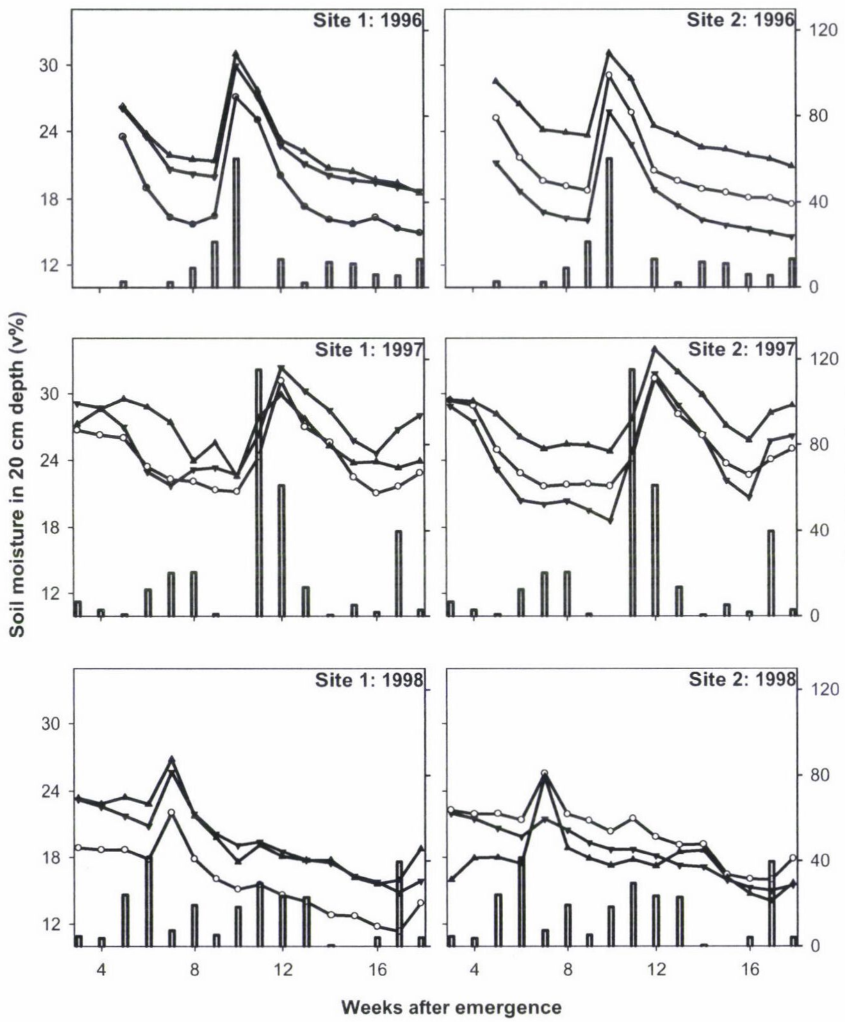

Fig. 1. Time course of soil moisture at a depth of 20 cm as affected by planting patterns and precipitation in the three years of testing. The symbols triangle down, circle and triangle up represent the planting patterns 12 plants m^{-2} at 50 cm row spacing, 8 plants m^{-2} at 75 cm row spacing and 4 plants m^{-2} at 50 cm row spacing, respectively. Precipitation is plotted as vertical bars

Except in 1998, planting patterns, precipitation and plant development had no effect on the soil moisture at 150 cm (data not shown), even though there were storms of 39.1 mm day^{-1} in July 1996 and 50.9 mm day^{-1} in July 1997. In 1998, the soil moisture at 150 cm decreased with plant development, which might have resulted from the response of plant growth to the early drought in this year. As suggested by Connor et al. (1985), pre-anthesis drought may increase the proportion of roots in deep soil layers. Precipitation in May 1998 was rare and the air temperature was abnormally high. Thus, the soil water at a depth of 150 cm might have been utilized in 1998. To summarize, in a normal year, e.g. 1997, sunflower crops in the region where the trials were conducted only use the soil water above 150 cm. In a dry year, particularly if the drought occurs in the early growth stages, e.g. 1998, soil water deeper than 150 cm may be depleted by sunflower crops. This is in line with previous findings (e.g. d'Andria et al., 1995) that sunflower can extract soil water to a depth of 180 cm or more in dry regions. These results should be taken into consideration when additional irrigation schedules are planned or field evapotranspiration is calculated.

Among the planting patterns, soil moisture changed more rapidly in RS1PD3 and RS2PD2 than in RS3PD1. The former two had almost the same values. There are various factors to explain the responses of soil moisture to planting patterns. First, dense populations in combination with narrow rows, e.g. RS1PD3 and RS2PD2, may enhance plant canopy development, as previously discussed. Canopy structure is the most important factor in balancing the partitioning of radiation between the plant and the soil surface. Thus, the amount of radiation reaching the ground, one of the most important factors determining changes in soil moisture at a high latitude site, can be altered considerably by planting patterns. Second, the root system, particularly the temporal and spatial distribution of the roots, may be modified by planting patterns (Long, 1999). Soil moisture can generally be changed by plant use and soil evaporation. In the present study, soil moisture was only monitored in the central row, where denser roots can be expected in narrow rows than in wide rows. Third, plant lodging differs among planting patterns, which may also affect soil moisture. Lodging tended to be higher in RS1PD3 and RS2PD2 than in RS3PD1 (Long, 1999).

Other factors may be the speed and direction of the wind, soil temperature, etc. The latter will be discussed in the next section, and here only the possible effect of wind will be outlined. The wind characteristics may modify the response of soil moisture to planting patterns, particular in the upper layer. At the present experimental sites, westerly and easterly winds prevailed (Long, 1999). Therefore, evaporation might be higher in east to west oriented rows (site 1) than in north to south oriented rows (site 2), since north to south oriented rows could break the prevailing winds. However, there is still doubt about the effect of wind on soil evaporation since the air in this region and latitude is mostly cool during the whole growing season. Furthermore, the soil in the central north to south rows might be much drier, since more rain water was observed to be intercepted by the canopy itself owing to the prevailing winds (data not shown). All in all, the situation is quite complicated; model simulations are required to elucidate the effects of planting patterns on soil moisture. Such models should combine the effects of latitude, wind, seasons and soil types on soil moisture.

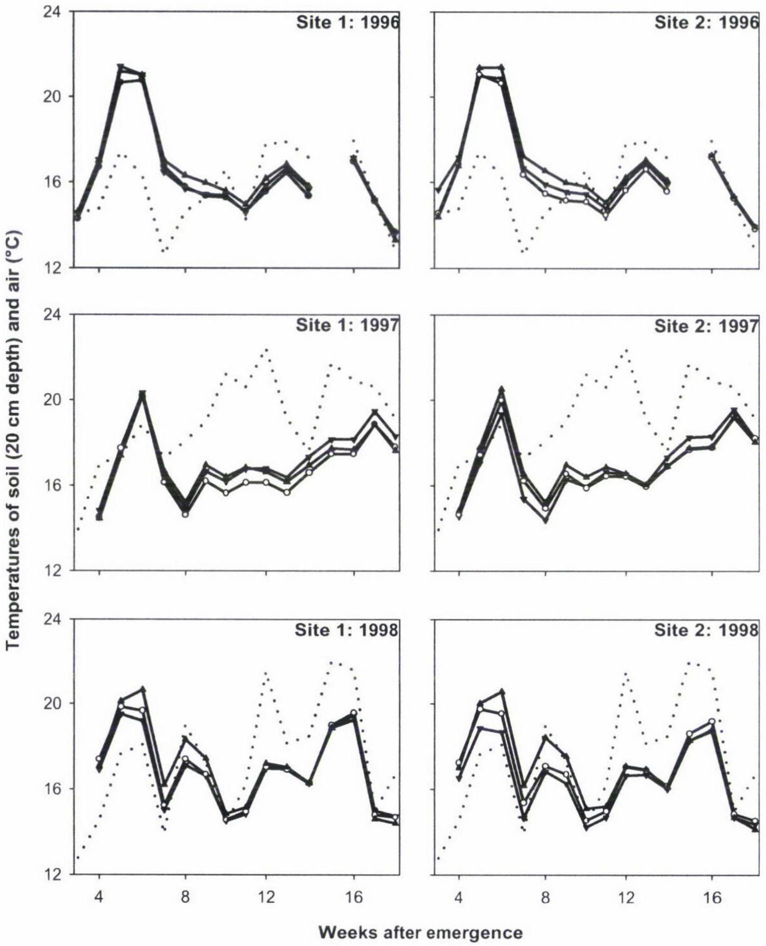

Fig. 2. Time course of soil temperature at a depth of 20 cm as affected by planting patterns and air temperature in the three years of testing. The symbols triangle down, circle and triangle up represent the planting patterns 12 plants m^{-2} at 50 cm row spacing, 8 plants m^{-2} at 75 cm row spacing and 4 plants m^{-2} at 100 cm row spacing, respectively. Air temperature is plotted as dotted lines

Seasonal variation of soil temperature

The soil temperature at a depth of 5 cm followed changes in the air temperature very closely in 1998 (data not shown). Before the closure of the whole canopy, the air temperature was lower than the soil temperature at 5 cm. The differences between air temperature and soil temperature at 5 cm became marginal from 7 to 13 weeks after emergence. The air temperature was higher than the soil temperature when the canopy opened again due to leaf senescence or lodging. Therefore, the soil is a good buffer for plant development, in particular during the early growth stage. The seasonal patterns of soil temperature in the ploughed layer (20 cm depth) fluctuated as did the air temperature. The amplitude of the fluctuation was smaller from 8 to 14 weeks after emergence in 1996 and 1997, and from 7 to 13 weeks after emergence in 1998 than during the early or late growth stages, when the leaf area index was more than 3 and the canopy was completely closed. In 1996, the soil temperature at 20 cm was 0.5 to 12.5°C higher than the respective air temperature. This was also true for 1998, but not for 1997. In 1998, the soil temperature at 20 cm was lower than at 5 cm, particularly when the soil temperature peaked.

The soil temperature at 50 cm was very variable and partly reflected the changes in air temperature. However, the responses of soil temperature at 50 cm to changes in air temperature were less pronounced than those at 5 and 20 cm. The soil at 50 cm was cooler than at 20 cm throughout the growing season. In addition, the temporal changes in soil temperature at 50 cm depth lagged behind those at 20 cm (data not shown). The soil temperature at 100 and 150 cm remained relatively constant (data not shown). The deepest layer investigated (150 cm depth) was cooler than the soil at 100 cm. These differences were more pronounced in 1997 than in 1996.

The soil temperature did not show consistent responses to planting patterns. Nevertheless, it could be seen that the soil temperature was mostly lower in RS1PD3 and RS2PD2 than in RS3PD1, particularly at a depth of 5 cm (data not shown). The former two had almost the same values. There are two possible reasons for this. First, it should be kept in mind that the temperature of a black soil (Haplic chernozem) depends mainly on the input of energy, i.e. the radiation penetrating to the ground. There is likely to be less penetrating radiation in RS1PD3 and RS2PD2 than in RS3PD1 due to rapid canopy development (Long, 1999). Second, the ability of the soil to reserve heat energy is high when the soil moisture is high. The results confirm this since soil moisture changed, for the most part, faster in RS1PD3 and RS2PD2 than in RS3PD1.

Conclusions

The development of sunflower crops at a high latitude (51°24' N) may be affected by planting patterns. However, the final dry matter accumulation hardly differed in the field trials and their simulation between the planting patterns (12 plants m^{-2} at 50 cm row spacing, 8 plants m^{-2} at 75 cm row spacing and 4 plants

m^{-2} at 100 cm row spacing). The canopy establishment during the growing season varied with the planting pattern, which might be partly responsible for changes in soil moisture at depths of 20, 50, 100 and 150 cm, and soil temperature at depths of 5, 20, 50, 100 and 150 cm. The most important factor to define the characteristics of soil moisture and soil temperature may be the amount of radiation penetrating to the ground. However, there may be an interaction between soil moisture and soil temperature, which could not be separated completely in the present study. Our study did confirm that the optimal production system for oilseed sunflower like Eurosol in central Germany seems to be 4 to 8 plants m^{-2} at 75 to 100 cm row spacing.

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IMPACT OF HERBICIDES AND THEIR APPLICATION TECHNIQUES ON YIELD AND RESIDUES IN COTTON-BASED INTERCROPPING SYSTEMS

A. VELAYUTHAM, A. MOHAMED ALI, V. VEERABADRAN and S. SANBAGAVALLI

DEPARTMENT OF AGRONOMY, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, INDIA

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Field experiments were conducted at the Agricultural College and Research Institute (Killikulam), Tamil Nadu Agricultural University from September 1992 to June 1995 to optimize the herbicide dose and application techniques for cotton-based intercropping systems. The performance of a sole cotton crop was compared with cotton + cowpea (2:2) and cotton + soybean (2:2) in combination with different weed management practices. The cotton + cowpea intercropping system had lower weed DMP (79.9 kg ha^{-1}) than cotton + soybean (88.5 kg ha^{-1}) or cotton sole crop (97.0 kg ha^{-1}) in both the years studied. Metolachlor at 1.0 kg ha^{-1} applied as herbigation (herbicide added to the irrigation water) registered the lowest total weed DMP of 31.9 kg ha^{-1} (1992–93) and 36.6 kg ha^{-1} (1994–95). With regard to weed smothering efficiency (WSE), the cotton + cowpea intercropping system had higher WSE (18.6%) than cotton + soybean (8.8%). The weeds present in the sole cotton crop removed more N, P and K at 40 DAS than at 20 DAS. In the cotton-based intercropping systems the removal of nutrients by weeds under unweeded conditions ranged from 7.8 to 8.9 kg ha^{-1} N, 0.5 to 0.68 kg ha^{-1} P and 4.38 to 6.78 kg ha^{-1} K at 20 DAS and 16.7–18.7 kg N ha^{-1}, 1.24–1.27 kg ha^{-1} P and 11.5–13.1 kg ha^{-1} K at 40 DAS. Sole cropping of cotton gave the highest seed cotton yield (1935 and 1789 kg ha^{-1}). The yield reduction in the cotton + cowpea system compared to sole cotton ranged from 40 to 125 kg ha^{-1}. The effect of intercropping soybean with cotton was less severe, with yields of 1893 and 1746 kg ha^{-1} (1992–93 and 1994–95, respectively) compared with 1815 and 1659 kg ha^{-1} for the cotton + cowpea system. The application of metolachlor at 1.0 kg ha^{-1} as herbigation led to the highest grain yields of 312 and 271 kg ha^{-1} in cowpea (1992–93 and 1994–95, respectively) and 465 and 410 kg ha^{-1} in soybean. The cotton + soybean system produced the highest net returns of 527.87 \$ and 509.20 \$ followed by cotton + cowpea (492.02 \$ and 462.67 \$). The interaction of the cotton + soybean system with the pre-emergence application of metolachlor at 1.0 kg ha^{-1} as herbigation + hand weeding produced the highest net returns (695.40 \$ and 688.20 \$). In the residue study, pendimethalin was found to leave higher amounts of residues in the seeds (0.0093 ppm) than metolachlor (0.0080 ppm).

Key words: cotton intercropping systems, weed management, weed DMP, weed smothering efficiency (WSE), weed index, residues

Introduction

Cotton is the most important fibre crop in the world, supporting a large textile industry and labour force. India ranks first in acreage among the cotton-growing countries, with an area of 7.55 million ha^{-1}, representing 23% of the world's cotton area and contributing 11.5% of the world's total production. The productivity of cotton in India is only 318 kg ha^{-1} as against a world average of 582 kg ha^{-1}. It is almost certain that an increase in the area under cotton will not

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be possible and increasing the productivity seems to be the only possible strategy. Since it is a wide-spaced, long duration crop, it provides ample scope for raising intercrops giving more effective resource utilization, higher total yields and enhanced income from unit area of land. To ensure sustainable additional income, short duration pulses, onion and other oilseed crops are traditionally intercropped with cotton. Thus, the crop is intensified in both time and space. Intercrops provide efficient coverage of the land, resulting in suppressed weed growth. Cotton is very sensitive to weed intensification due to its slow initial growth, while wider spacing gives a better chance for weed infestation. Yield loss due to uncontrolled weed infestation was reported to be as much as 50 to 85%. Mechanical or cultural methods of weed control, though used widely, are laborious, time-consuming and expensive on account of the scarcity of labour. Therefore, chemical weed control provides an alternative solution in times of labour scarcity.

Weeds remove 30–50% of applied fertilizer and 20–40% moisture, besides reducing the yield and quality of the produce (Subramanian et al., 1991). Jain et al. (1981) found that weeds removed 5–6 times more N, 5–12 times more P and 2–5 times more K than the cotton crop at the early stages. The removal of nutrients by weeds was less in intercropping than in the sole crop of cotton. The weed flora and its competitiveness with crops varies widely in different cropping systems. The herbicides chosen should be selective for the main and component crops, as well as having little or no residual effect on succeeding crops. For effective weed control, the uniform application of herbicide is very important. Among the herbicide application techniques, spraying, sand mixes and herbigation are widely used. Although much research work has been done on both intercropping systems and weed management practices, there is a paucity of information about the effect of herbicide application techniques in intercropping systems.

The objective of this study was to evaluate the suitability of pre-emergence herbicides and their application techniques in cotton-based intercropping systems and to study the effect of applied herbicides to cotton in the soil and its residues in the harvested products of the intercrops.

Materials and methods

Field experiments were conducted at the Agricultural College and Research Institute (Killikulam), Tamil Nadu Agricultural University during the winter season (September–February) for two years, in 1992–1993 and 1994–1995. Sorghum was raised as a residual crop during the summer season (February–June) of 1993 and 1995. The soil texture was red sandy loam, classified as Typic Rhodostaulf with a pH of 6.6–7.5. The soil was medium in available N (278 kg ha^{-1}), low in phosphorus (26 kg ha^{-1}) and high in potassium (491 kg ha^{-1}).

Treatment details

Main plots (Cropping systems – C)
C_1 – Cotton sole crop
C_2 – Cotton + cowpea (2:2)
C_3 – Cotton + soybean (2:2)

Subplots (weed management – W)
W_1 – Metolachlor at 0.75 kg ha^{-1} as herbigation
W_2 – Metolachlor at 1.00 kg ha^{-1} as herbigation
W_3 – Metolachlor at 0.75 kg ha^{-1} as spray
W_4 – Metolachlor at 1.00 kg ha^{-1} as spray
W_5 – Pendimethalin at 0.75 kg ha^{-1} as herbigation
W_6 – Pendimethalin at 1.00 kg ha^{-1} as herbigation
W_7 – Pendimethalin at 0.75 kg ha^{-1} as spray
W_8 – Pendimethalin at 1.00 kg ha^{-1} as spray
W_9 – Hand hoeing and weeding at 20 and 40 DAS
W_{10} – Unweeded check
One hoeing and weeding was given to all the herbicide treatments (W_1 to W_8) at 40 DAS.

The cotton variety MCU 11, the cowpea variety CO 4, and the soybean variety CO 1 were used. The field was ploughed and levelled, beds and channels were formed, and the cotton seeds were sown in paired rows of 60 × 30 cm. In the case of sole cotton, the seeds were sown in normal rows at a spacing of 75 × 30 cm. The intercrops, namely cowpea and soybean, were sown in two rows in between the pairs of cotton rows by adopting a spacing of 30 × 10 cm, after inoculating the seeds with rhizobium. 80:40:40 kg NPK ha^{-1} was applied to the cotton crop as urea, superphosphate and muriate of potash, respectively. Basal dressing of 40:40:40 kg NPK ha^{-1} was applied and 40 kg N ha^{-1} was applied at 45 DAS.

For the herbigation technique, a saline drip bottle (500 ml) was filled with herbicide without dilution. This was hung downwards at a height of 1.5 m in front of the irrigation channel inlet to the plots in order to drip the herbicide droplets continuously on top of the flowing irrigation water. The quantity of herbicides required per unit area was calibrated considering the quantum of water flow. The herbicides were applied along with life irrigation (second irrigation after the first irrigation on the day of sowing) (3 DAS) in the case of herbigation. For herbicide as spray, the treatment was done at 3 DAS followed by life irrigation. In all herbicide-treated plots one hoeing and weeding was given at 40 DAS. Observations on weed DMP and weed smothering efficiency (WSE) were recorded.

Weed Smothering Efficiency (WSE)

$$\text{WSE} = \frac{W_o - W_t}{W_o} \times 100$$

where: W_o – Weed dry weight in the sole crop; W_t – Weed dry weight in the intercropping systems

Weed Index (WI) Gill and Vijayakumar (1969)

$$\text{WI} = \frac{X - Y}{X} \times 100$$

where: X – Yield from minimum weed competition; Y – Yield from treated plot for which WI is to be calculated

Metolachlor at 1.00 kg ha^{-1} as herbigation + hand weeding was taken as the base for computing the weed index.

The seed cotton was harvested in four pickings at weekly intervals. The seed cotton yield obtained from the net plot area at each picking was pooled after shade drying and the total weight was recorded and expressed in kg ha^{-1}. The quantities of metolachlor and pendimethalin residues in the soil (1–15 cm depth) and the seeds were analysed using gas chromatography (Sankaran et al., 1993).

Results and discussion

Effect of weed management practices and intercropping systems on weed dry matter production (DMP) and weed smothering efficiency (WSE)

The cotton + cowpea intercropping system gave lower weed DMP (79.9 kg ha^{-1}) than cotton + soybean (88.5 kg ha^{-1}) and cotton sole crop (97.0 kg ha^{-1}) in the years studied. This is natural due to the weed suppression and/or smothering efficiency of the intercrops through the canopy cover and higher population pressure, which led to a reduction in the weed density. Jayaraj (1991) pointed out that the weed suppression in intercropping was due to the increased population per unit area and the crop competition for natural resources. A greater reduction in weed DMP in intercropping was also reported by Prasad and Srivastava (1991).

At 20 DAS the application of metolachlor and pendimethalin reduced the total weed DMP (74.8 and 80.6 kg ha^{-1} for 1992–93 and 1994–95) significantly over hand weeding twice (82.8 and 89.4 kg ha^{-1}, respectively) and the unweeded check (169.3 and 183.6 kg ha^{-1}). Metolachlor at 1.0 kg ha^{-1} as herbigation registered the lowest total weed DMP of 31.9 kg and 36.6 kg ha^{-1}, respectively, followed by metolachlor at 1.00 kg ha^{-1} as spray (39.4 and 43.8 kg ha^{-1}), which was on par with metolachlor at 0.75 kg ha^{-1} as herbigation. At 40 DAS, the highest total weed DMP was recorded in the unweeded check (460.5 kg ha^{-1}), compared with 127.8 kg ha^{-1} in the metolachlor and pendimethalin plots and 165.8 kg ha^{-1} for handweeding twice. Metolachlor at 1.0 kg ha^{-1} as herbigation reduced the total weed DMP significantly (86.0 and 97.2 kg ha^{-1}), followed by metolachlor at 1.0 kg ha^{-1} as spray (99.1 and 111.1 kg ha^{-1}). Cotton + cowpea intercropping treated with metolachlor at 1.0 kg ha^{-1} as herbigation gave the lowest total weed DMP, followed by metolachlor at 1.0 kg ha^{-1} as spray, which was comparable with metolachlor at 0.75 kg ha^{-1} as herbigation.

The cotton + cowpea intercropping system resulted in higher weed smothering efficiency (18.6%) than the cotton + soybean (8.8%) system in both the years. In the cotton + cowpea intercropping system, the WSE of cowpea at 20 DAS was only 8.6–9.2%, while higher values ranging from 14.8–24.9 % were recorded after the pre–emergence application of metolachlor (Table 1). Similarly, in the case of cotton + soybean intercropping the WSE at 20 DAS in the unweeded check was only 4.2–4.6%, whereas it was higher (7.6–15.4) after metolachlor application. The weed smothering efficiency of the intercropping system was higher at 40 DAS (22.2% for the cotton + cowpea system and 9.6% for the cotton + soybean system) than at 20 DAS (8.1% for cotton + soybean and 15.5% for cotton + cowpea). At 40 DAS the improvement in the weed smothering efficiency of the intercropped canopy due to weed management practices was less distinct. At this stage the smothering effect was greater due to the fully developed canopy of the intercrops. Among the weed management practices studied, metolachlor at 1.0 kg ha^{-1} as herbigation led to the highest WSE of 16.4% at 20 DAS and 23.0% at 40 DAS. The unweeded check performed less well (6.7% at 20 DAS and 13.9% at 40 DAS) than the other treatments.

Table 1
Weed smothering efficiency (%) of different intercropping systems

Treatments	1992–1993				1994–1995			
	20 days		40 days		20 days		40 days	
	C+C*	C+S	C+C	C+S	C+C	C+S	C+C	C+S
W_1	19.3	8.2	26.5	10.8	17.2	8.1	25.5	11.5
W_2	24.9	15.4	30.1	12.4	16.0	9.3	32.2	17.2
W_3	20.1	7.6	24.2	10.0	14.8	7.7	26.2	11.7
W_4	19.8	8.3	27.5	10.9	17.4	7.9	27.2	12.5
W_5	16.3	10.8	18.9	7.5	11.4	6.8	19.9	9.9
W_6	19.5	10.0	20.1	8.2	14.2	7.2	21.0	10.3
W_7	18.2	9.4	16.6	5.4	12.7	6.3	16.5	7.1
W_8	16.7	10.9	19.0	6.9	11.5	6.9	20.3	9.4
W_9	10.7	5.9	16.1	5.2	10.4	5.6	18.0	7.3
W_{10}	9.2	4.6	17.6	8.0	8.6	4.2	20.1	10.0
Mean	16.4	8.9	21.7	8.5	13.3	6.8	22.7	10.7

*C+C: Cotton+cowpea; C+S: Cotton+soybean

Effect of intercropping systems and weed management practices on nutrient uptake of weeds and weed index (WI)

Metolachlor at 1.0 kg ha^{-1} as herbigation + hand weeding was taken as the base for computing WI (Table 2). In both the years metolachlor at 1.0 kg ha^{-1} as spray + hand weeding application gave the lowest weed index of 3.9 and 3.8%, followed by the same herbicide at 0.75 kg ha^{-1} as herbigation + hand weeding (5.6 and 5.1% in the first and second years, respectively). The unweeded check had a weed index of 66.7 and 69.2%, while that of hand weeding twice was only 22.0 and 23.0%.

In cotton-based cropping systems the removal of nutrients by weeds under unweeded conditions ranged from 7.8 to 8.9 kg ha^{-1} nitrogen (N), 0.5 to 0.68 kg ha^{-1} phosphorus (P) and 4.38 to 6.78 kg ha^{-1} potassium (K) at 20 DAS and 16.7–18.7 kg N ha^{-1}, 1.24–1.27 kg ha^{-1} P and 11.5–13.1 kg ha^{-1} K at 40 DAS (Table 3). The weeds present in the sole cropping of cotton removed more N, P and K at 40 DAS than at 20 DAS. Among the intercropping systems, the cotton + soybean intercropping system resulted in greater nutrient depletion by weeds than the cotton + cowpea system. Mohamed Ali et al. (1987) stated that the removal of nutrients by weeds was significantly lower in cotton-based intercropping systems with green gram than with onion.

Metolachlor at 1.0 kg ha^{-1} as herbigation resulted in the lowest N removal by weeds at 20 DAS (1.51 and 1.61 kg ha^{-1}) followed by metolachlor at 1.0 kg ha^{-1} as spray (1.60 and 1.79 kg ha^{-1}), which was on par with metolachor at 0.75 kg ha^{-1} as herbigation (1.66 and 1.85 kg ha^{-1}). At 40 DAS, all the weed management practices, including hand weeding twice, significantly lowered the N depletion by weeds over the unweeded check (Table 3). The interaction of herbicides and intercropping systems was significant at 40 DAS. In all the cropping systems, metolachor at 1.0 kg ha^{-1} as herbigation caused less depletion of N by weeds compared with the unweeded check.

Table 2
Effect of cropping systems and weed management practices on weed DMP and WI

Treatments	Weed dry matter production (DMP) (kg ha^{-1})				Weed index (WI)	
	1992–1993		1994–1995		1992–93	1994–95
	20 days	40 days	20 days	40 days		
Cropping systems (C)						
C_1	101.2 (1.861)	172.8 (2.197)	108.9 (1.895)	193.3 (2.248)	20.1	20.5
C_2	86.6 (1.781)	137.8 (2.093)	96.6 (1.834)	151.5 (2.139)	17.7	19.4
C_3	94.0 (1.821)	158.8 (2.159)	102.7 (1.864)	173.5 (2.196)	19.6	20.1
CD	0.003	0.006	0.011	0.020		
Weed management practices (W)						
W_1	39.9 (1.621)	102.7 (2.017)	44.2 (1.664)	114.7 (2.074)	5.6	5.1
W_2	31.9 (1.528)	86.0 (1.939)	36.6 (1.586)	97.2 (1.991)	*	*
W_3	44.2 (1.663)	114.7 (2.064)	49.3 (1.709)	126.9 (2.107)	9.1	9.6
W_4	39.4 (1.616)	99.1 (2.001)	43.8 (1.659)	111.1 (2.050)	3.9	3.8
W_5	49.6 (1.711)	143.6 (2.161)	55.2 (1.757)	157.3 (2.217)	18.4	18.6
W_6	46.2 (1.681)	129.6 (2.118)	51.6 (1.729)	141.5 (2.155)	13.0	13.5
W_7	53.3 (1.741)	157.0 (2.202)	58.3 (1.779)	170.7 (2.236)	21.3	21.3
W_8	49.0 (1.706)	140.2 (2.151)	54.7 (1.753)	152.6 (2.187)	16.6	16.7
W_9	283.8 (2.455)	158.5 (2.204)	303.6 (2.485)	173.0 (2.242)	22.0	22.3
W_{10}	305.1 (2.487)	433.4 (2.638)	329.6 (2.520)	487.5 (2.683)	66.7	69.2
CD	0.010	0.012	0.016	0.020		
CD (C×W)	0.018	0.020	NS	0.036		

* Base for computing weed index; Values in parenthesis –log x+2 transformation; NS – Non-significant

In both the years, metolachlor at 1.0 kg ha^{-1} as herbigation and hand weeding twice led to lower P depletion by weeds and were comparable with each other. The interaction effect was significant at 20 DAS but not at 40 DAS in the second year. The cotton + cowpea intercropping system with hand weeding twice resulted in less P removal by weeds, and this was comparable with metolachor at 1.0 kg ha^{-1} as herbigation.

Table 3
Nutrient uptake (kg ha^{-1}) as influenced by intercropping and weed management practices in cotton

T⁺	20 days after sowing						40 days after sowing					
	1992–93			1994–95			1992–93			1994–95		
	N	P	K	N	P	K	N	P	K	N	P	K
Cropping system (C)												
C_1	3.10	0.198	1.414	3.54	0.255	2.161	5.87	0.495	4.78	6.44	0.537	5.25
C_2	2.99	0.183	1.354	3.19	0.237	1.905	4.95	0.446	4.23	5.61	0.466	4.62
C_3	3.07	0.190	1.373	3.33	0.243	1.944	5.09	0.454	4.38	5.79	0.478	4.78
CD	0.08	0.008	0.039	0.11	0.011	0.039	0.08	0.025	0.05	0.08	0.008	0.11
Weed management practices (W)												
W_1	1.66	0.109	0.715	1.85	0.135	0.914	3.97	0.349	3.44	4.15	0.380	3.65
W_2	1.51	0.075	0.654	1.61	0.116	0.875	3.21	0.311	3.20	3.55	0.352	3.40
W_3	1.84	0.122	0.728	2.05	0.148	0.936	3.94	0.375	3.57	4.47	0.402	3.84
W_4	1.60	0.100	0.709	1.79	0.130	0.909	3.55	0.339	3.38	4.01	0.373	3.59
W_5	2.34	0.152	0.798	2.44	0.179	1.006	4.56	0.429	4.15	5.17	0.453	4.29
W_6	2.02	0.135	0.774	2.25	0.161	0.981	4.21	0.401	3.83	4.75	0.424	4.03
W_7	2.51	0.165	0.821	2.61	0.189	1.033	4.87	0.455	4.30	5.49	0.478	4.49
W_8	2.25	0.147	0.802	2.39	0.174	1.000	4.43	0.422	4.10	5.06	0.445	4.23
W_9	7.05	0.396	3.418	7.56	0.536	5.594	3.60	0.332	3.22	4.09	0.354	3.52
W_{10}	7.78	0.499	4.382	8.96	0.684	6.783	16.69	1.236	11.46	18.71	1.273	13.77
CD	0.14	0.012	0.060	0.14	0.016	0.078	0.20	0.028	0.12	0.18	0.014	0.16
CD*	NS	NS	NS	0.24	NS	0.134	0.34	NS	0.22	0.32	NS	0.26

⁺T: Treatments; NS – Non-significant; *(C×W)

The uptake of K by weeds at 20 and 40 DAS varied significantly for the different cropping systems. At 20 DAS, herbicide application caused less K depletion by weeds compared with hand weeding twice, and the unweeded check. Metolachlor at 1.0 kg ha^{-1} as herbigation reduced the K removal by weeds and was comparable with the same herbicide as spray. Due to the effective control of weeds by the different weed management practices adopted, it eliminates the competition for nutrients by weeds. At 40 DAS, all the weed management practices, including hand weeding twice significantly reduced the K uptake by weeds over the unweeded check. The cotton + cowpea intercropping system with metolachlor at 1.0 kg ha^{-1} as herbigation showed the lowest uptake by weeds and was comparable with hand weeding twice and metolachlor at 1.0 kg ha^{-1} as spray.

Effect of intercropping systems and weed management practices on yield (kg ha^{-1}) and net returns

The sole cropping of cotton gave the highest seed cotton yield in both the years (1935 and 1789 kg ha^{-1}). The yield reduction in the cotton + cowpea system compared with sole cotton ranged from 40 to 125 kg ha^{-1}. The effect of intercropping soybean with cotton was less severe, with yields of 1899 and 1746 kg ha^{-1} in 1992–93 and 1994–95, than the cotton + cowpea system (1815 and

1659 kg ha^{-1}). The higher yield in the cotton + soybean system was due to the better yield attributes of cotton than in the cotton + cowpea system. Mishra et al. (1990) stated that the pre-emergence application of herbicides effectively controlled the weeds at critical periods (30–40 DAS) and thus promoted soybean growth and yield. Prakash et al. (1991) obtained similar results in soybean with metolachlor. Weed management practices caused a significant variation in the seed cotton yield. The unweeded check had the lowest seed cotton yields of 761 and 652 kg ha^{-1} in both the years. On overall comparison, metolachlor + hand weeding was superior to pendimethalin + hand weeding and handweeding twice (Table 4). The effectiveness of metolachlor in providing good early control of grasses, sedges and broad-leaved weeds allowed the crops to take up more nutrients and exhibit better growth and yield. The sole cotton system with metolachlor at 1.0 kg ha^{-1} as herbigation + hand weeding proved to be the best combination for seed cotton yield. Other authors (AICRPWC, 1993) reported similar findings with metolachlor application (Table 4).

The grain yield of cowpea was very poor (80 and 40 kg ha^{-1}) in the two years in the unweeded check. Weed management practices involving herbicides and hand weeding twice increased the yield by 117–229 kg ha^{-1}. The application of metolachor at 1.0 kg ha^{-1} as herbigation led to the highest grain yields of 312 and 271 kg ha^{-1} in 1992–93 and 1994–95, respectively. This was followed by the same chemical as spray (291 to 247 kg ha^{-1}), which was comparable with metolachor at 0.75 kg ha^{-1} as herbigation (286 and 240 kg ha^{-1}). The application of pendimethalin at 0.75 kg ha^{-1} as spray was on par with hand weeding twice in both years. Weed management practices improved the soybean yield by 243–358 kg ha^{-1} in the first year and 214–314 kg ha^{-1} in the second year as against the lowest yields of 107 and 96 kg ha^{-1} in the unweeded check. Metolachlor at 1.0 kg ha^{-1} as herbigation resulted in the highest grain yields of 467 and 410 kg ha^{-1}, followed by the same herbicide at 1.0 kg ha^{-1} as spray and at 0.75 kg^{-1} as herbigation.

With regard to net returns (Table 4) the cotton + soybean system gave the highest net returns of 527.85 $ and 509.20 $ in the two years, followed by the cotton + cowpea system (492.02 $ and 462.67 $). The sole cotton system recorded net returns of 445.22 $ and 442.65 $ during 1992–93 and 1994–95, respectively. Muthusankaranarayanan et al. (1989) and Patel et al. (1995) also reported that though the seed cotton yield was reduced due to intercropping with soybean and cowpea the economic returns were higher due to the additional yield of soybean or cowpea. Among the different weed management practices studied, the highest net returns of 649.38 $ and 641.73 $ were obtained with metolachlor at 1.0 kg ha^{-1} as herbigation in all the cropping systems. Handweeding twice gave net returns of 449.95 $ and 427.87 $. Additional returns of 378.59 $ and 570.24 $/ha were obtained by adopting different weed management practices compared with the unweeded check. The interaction effect of the cotton + soybean system with the pre-emergence application of metolachlor at 1.0 kg ha^{-1} as herbigation + hand weeding twice led to the highest net returns (695.4 $ and 688.2 $).

Table 4
Effect of weed management practices and intercropping systems on yield (kg ha^{-1}) and economics

Treatments	Seed cotton yield		Soybean yield		Cowpea yield		Net returns ($)	
	1992–93	1994–95	1992–93	1994–95	1992–93	1994–95	1992–93	1994–95
Cropping systems (C)								
C_1	1935	1789	–	–	–	–	445.22	442.65
C_2	1815	1659	–	–	–	–	492.02	462.67
C_3	1899	1746	–	–	–	–	527.85	509.20
CD	19	25	–	–	–	–	–	
Weed management practices (W)								
W_1	2159	2006	434	389	286	240	601.62	595.27
W_2	2287	2111	467	410	312	271	649.37	641.73
W_3	2078	1912	411	366	263	219	563.41	553.16
W_4	2197	2033	446	390	291	247	611.82	603.77
W_5	1865	1719	373	322	223	169	477.91	460.78
W_6	1989	1826	397	350	244	196	521.55	504.58
W_7	1800	1660	357	311	205	142	449.18	431.00
W_8	1907	1758	385	336	225	175	487.45	471.91
W_9	1784	1641	350	310	200	133	449.94	427.87
W_{10}	761	652	107	96	80	40	71.36	25.03
CD	64	54	21	17	15	10	–	–
C × W	102	90	–	–	–	–	–	–

Effect of weed management practices on herbicide residues in the soil and in cotton, cowpea and soybean seeds

Generally the herbicide residues in the soil samples were very low compared to the prescribed maximum residual limit (MRL) values (0.0067 and 0.0078 ppm for the two years). Among the different plant parts, higher residual values of both the herbicides were observed in soybean grain (0.0115 to 0.0097 ppm for 1992–93 and 1994–95) than in cowpea (0.0106 and 0.0092 ppm) and cotton seed (0.0049 and 0.0042 ppm), but these were below the MRL values. Among the two levels of metolachlor, 0.75 kg ha^{-1} left a non-detectable level of residues in cotton seed and soil. The application of higher doses of the herbicides left higher amounts of residues in the soil compared to the lower doses. Pendimethalin left a higher amount of residues in the seeds (0.0093 ppm) than metolachlor (0.0080 ppm) (Table 5).

The results of the two-year study indicated that the intercropping system with cotton + cowpea gave lower weed DMP and higher WSE than the cotton + soybean system. Nutrient removal by weeds was higher in sole cotton cropping than in the intercropping systems studied. The seed cotton yield reduction in the cotton + cowpea system compared with sole cotton was 40 to 125 kg ha^{-1}. The cotton + soybean system led to better yields (1823 kg ha^{-1}) than cotton + cowpea (1737 kg ha^{-1}). The application of metolachlor at 1.0 kg ha^{-1} as herbigation led to the highest cowpea and soybean yields and net returns. The interaction of cotton + soybean with the pre-emergence application of metolachlor at 1.0 kg ha^{-1} as herbigation + hand weeding twice resulted in the highest net returns.

Table 5
Residues of metolachlor and pendimethalin in soil, cotton seed, cowpea grain and soybean grain (ppm detected)

Treatments	1992–1993				1994–1995			
	Soil	Cotton seed	Cowpea grain	Soybean grain	Soil	Cotton seed	Cowpea grain	Soybean grain
W_1	ND	ND	0.0082	0.0098	ND	ND	0.0062	0.0071
W_2	0.0019	0.0012	0.0108	0.0111	0.0026	0.0018	0.0082	0.0102
W_3	ND	ND	0.0094	0.0101	ND	ND	0.0082	0.0080
W_4	0.0023	0.0016	0.0118	0.0123	0.0028	0.0021	0.0107	0.0104
W_5	0.0076	0.0041	0.0099	0.0101	0.0081	0.0053	0.0089	0.0093
W_6	0.0098	0.0068	0.0113	0.0136	0.0099	0.0071	0.0102	0.0106
W_7	0.0081	0.0043	0.0107	0.0108	0.0087	0.0049	0.0098	0.0099
W_8	0.0104	0.0069	0.0125	0.0140	0.0110	0.0080	0.0114	0.0120
W_9	–	–	–	–	–	–	–	–
W_{10}	–	–	–	–	–	–	–	–

Maximum Residue Limit (ppm):

	Soil	Cotton seed	Cowpea grain	Soybean grain
Metolachlor:		0.1	NA	0.1
Pendimeth.	0.1	NA	NA	

ND: Non-detectable; NA: Not available; Pendimeth.: Pendimethalin

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Short communication

EFFECT OF SOWING DATE ON GRAIN YIELD OF CRAB GRASS, *Digitaria* spp.

S. O. BAKARE[1], M. G. M. KOLO[2] and J. A. OLADIRAN[2]

[1]NATIONAL CEREALS RESEARCH INSTITUTE, BIDA, NIGER STATE, NIGERIA
[2]DEPARTMENT OF CROP PRODUCTION, SCHOOL OF AGRICULTURE AND AGRICULTURAL TECHNOLOGY, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA

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There was a significant interaction effect between the variety and the sowing date for the number of productive tillers, indicating that the response to sowing date varied with the variety. A significant reduction in the number of productive tillers became evident when sowing was delayed till 26 June in the straggling variety as compared to sowing dates in May. Lower numbers of productive tillers were also recorded when the sowing of the erect variety was further delayed till 10 July. The grain yield data showed that it is not advisable to sow the straggling variety later than 12 June, while sowing may continue till about 26 June for the erect variety in the study area.

Key words: crab grass, *Digitaria*, grain yield, sowing date

Introduction

Farmers in Nigeria use a wide range of planting dates for crab grass (*Digitaria* spp.) ranging from May to July for both erect and straggling varieties. However, the variation in the maturity periods of the erect and straggling varieties is an important factor which should be considered in crab grass production. Straggling varieties mature earlier than erect ones. The maturity period for erect varieties is about 130 days, while that of the straggling type ranges between 90–100 days (Purseglove, 1975; Wrigley, 1981). The time of sowing crops has an influence on the grain yield (Havlicek, 1985; Petr, 1991). It is therefore necessary to determine the appropriate sowing date for each of the varieties.

Materials and methods

A 2 × 6 factorial experiment was carried out in the 1997 and 1998 wet seasons (May–October) at the experimental site of the National Cereals Research Institute, Badeggi, Nigeria (9° 45'N, 6° 7'E) to determine the effect of different sowing dates on the yield of crab grass. The mean monthly meteorological data for 1997 and 1998 are indicated in Table 1. The first factor was variety while the second was sowing date. There were six sowing dates, two in each month of May–July (15 May, 29 May, 12 June, 26 June, 10 July and 24 July) and two varieties of crab grass (Abuut, an erect variety and Ex-Sum, a straggling variety). There was thus a total of 12 treatment combinations replicated three times. Land preparation was done using the conventional ploughing and harrowing on 5 and 13 May, 1997 and on 10 and 13 May, 1998, respectively. The sowing of each variety was done by broadcasting the seeds on the flat on each sowing date. For each gross plot size of 5 m × 4 m, a seed rate of 25 kg/ha was sown. The data obtained included: days to 50% flowering, number of productive tillers and grain yield at 14% moisture content. Analysis of variance was carried out on the data using MSTATC software and the means obtained were compared using Duncan's New Multiple Range Test.

Table 1
Mean monthly meteorological data for 1997 and 1998

Month	Rainfall (mm)	Temperature		Relative humidity (%)
		Max.	Min.	
1997				
Jan	0.0	36	22	57
Feb	0.0	37	20	30
Mar	64.9	38	23	54
Apr	53.9	36	24	70
May	129.3	34	23	77
Jun	279.2	32	22	82
Jul	219.0	32	23	84
Aug	227.2	32	23	82
Sep	145.7	32	23	83
Oct	135.4	33	23	82
Nov	0.0	36	21	72
Dec	0.0	35	17	57
Total	1254.6	413	264	830
Mean		34	22	69
1998				
Jan	0.0	35	17	52
Feb	Trace	39	21	43
Mar	0.0	40	23	33
Apr	67.1	39	27	68
May	213.3	34	25	82
Jun	75.5	33	24	82
Jul	239.7	32	24	85
Aug	145.5	30	24	84
Sep	153.7	31	23	85
Oct	103.0	33	24	82
Nov	0.0	36	20	74
Dec	0.0	35	17	59
Total	997.7	417	269	829
Mean		35	22	69

Source: National Cereals Research Institute, Meteorological Unit, Badeggi, Nigeria.

Results and discussion

An interaction effect was found between variety and sowing date for the number of days to 50% flowering in both years at the 5% level of significance (Table 2). The number of days to 50% flowering in the erect variety was significantly greater than that of the straggling variety, ranging between 70 and 85 in the straggling variety and between 96 and 111 in the erect in 1997, with 76–90 and 100–115 for the straggling and erect varieties, respectively, in 1998. The use of different varieties and the adoption of different sowing dates resulted in a significant difference in the days to 50% flowering. It was observed that plants sown earlier flowered later than those sown at later dates.

There were significant differences in the number of productive tillers in both years (Table 3). Earlier sowing dates (15 May to 12 June) gave more productive tillers than later sowing dates for the straggling variety. For the erect variety, fewer productive tillers were obtained when sowing was carried out later than 10 July. The highest number of productive tillers (31 per hill) was obtained when the straggling variety was sown between 15 May and 12 June in 1997, while the erect variety produced a maximum of eight tillers per hill in the same year. A similar trend was observed in 1998, with 30 productive tillers per hill for the straggling variety and 6 per hill for the erect. The significant interaction effect is an indication that the response to sowing date varied with the variety. Whereas a significant reduction in the productive tiller count became evident when sowing was delayed till 26 June in the straggling variety, a decline compared to the May values was only recorded for the erect variety when sowing of was further delayed till 10 July in both years.

Table 2

Effect of sowing date on days to 50% flowering of crab grass types in 1997 and 1998

Sowing date	1997			1998		
	Straggling	Erect	Mean	Straggling	Erect	Mean
15 May	85.0^{d}	111.3^{a}	98.2^{a}	90.0^{d}	115.3^{a}	102.5^{a}
29 May	80.0^{e}	110.0^{a}	95.0^{b}	90.0^{d}	114.0^{a}	102.0^{a}
12 June	79.0^{ef}	106.7^{b}	92.9^{b}	85.0^{e}	110.0^{b}	97.5^{b}
26 June	78.0^{ef}	96.3^{c}	87.2^{c}	80.0^{f}	110.0^{b}	95.0^{b}
10 July	76.7^{f}	96.3^{c}	86.5^{c}	78.0^{fg}	100.0^{c}	89.0^{c}
24 July	70.0^{g}	96.0^{c}	83.0^{d}	76.2^{g}	100.0^{c}	88.1^{c}
Mean	78.1^{b}	102.8^{a}		83.2^{b}	108.2^{a}	
SE±						
Variety		0.6			0.8	
Sowing date		1.1			1.5	
Interaction		1.6			1.8	
CV%		2.1			8.5	

Means with the same letter(s) in the same column are not significantly different at the 5% level of significance.

Table 3

Influence of sowing date on the number of productive tillers of crab grass types in 1997 and 1998

Sowing date	1997			1998		
	Straggling	Erect	Mean	Straggling	Erect	Mean
15 May	31.0^{a}	7.7^{d}	19.4^{a}	30.0^{a}	6.0^{d}	18.0^{a}
29 May	31.0^{a}	7.7^{d}	19.4^{a}	30.0^{a}	6.0^{d}	18.0^{a}
12 June	31.3^{a}	7.3^{de}	19.3^{a}	30.0^{a}	6.0^{d}	18.0^{a}
26 June	28.3^{b}	7.3^{de}	17.8^{a}	27.5^{b}	6.0^{d}	16.8^{a}
10 July	15.0^{c}	5.0^{e}	10.0^{b}	18.5^{c}	4.0^{d}	11.3^{b}
24 July	15.0^{c}	4.0^{e}	9.5^{b}	18.0^{c}	4.0^{d}	11.0^{b}
Mean	25.3^{a}	6.5^{b}	-	25.7^{a}	5.3^{b}	-
SE±						
Variety		0.5			0.6	
Sowing date		0.8			0.8	
Interaction		1.2			1.1	
CV%		9.8			15.0	

Means with the same letter(s) in the same column are not significantly different at the 5% level of significance.

The grain yield ranged from 33–485 kg/ha in the straggling variety and from 63–243 kg/ha in the erect type in 1997 (Table 4), and from 80–475 kg/ha in the straggling variety and 70–300 kg/ha in the erect type in 1998. In both varieties, the grain yield was high when sowing took place in May or June, while drastic reductions were only observed when the crop was sown in July. The grain yield data showed that there was no significant difference in sowing dates between 15 May and 12 June for the straggling variety and 15 May–26 June for the erect type. Sowing dates from 26 June onward gave significantly lower yields for the straggling variety, while the reduction in yield of the erect variety was more evident for sowing dates from 10 July onwards. There was a relationship between the number of productive tillers and the grain yield at each sowing date. A larger number of productive tillers produced more grains, as indicated in the regression analysis. The regression analysis between the productive tillers and grain yield gave the formulas $Y = -291.9 + 22.6x$ and $Y = -342.1 + 25.5x$ for the straggling variety in 1997 and 1998, respectively. and $Y = -109.7 + 41.2x$ and $Y = -245.5 + 82.6x$ for the erect variety in 1997 and 1998, respectively. The yield values indicated that it is not advisable to go beyond 12 June when sowing the straggling variety, while sowing may continue till about 26 June for the erect variety in the study area.

Table 4

Effect of sowing date on the grain yield (kg/ha) of crab grass types in 1997 and 1998

Sowing date	1997			1998		
	Straggling	Erect	Mean	Straggling	Erect	Mean
15 May	400.0^{a}	175.0^{bc}	287.5^{ab}	405.0^{ab}	200.5^{bc}	302.8^{b}
29 May	433.0^{a}	156.7^{bc}	294.9^{ab}	430.3^{a}	200.4^{bc}	315.4^{b}
12 June	485.0^{a}	243.3^{b}	364.2^{a}	475.5^{a}	300.0^{b}	387.8^{a}
26 June	246.7^{b}	240.0^{b}	243.4^{b}	300.0^{b}	300.0^{b}	300.0^{b}
10 July	80.0^{c}	70.0^{c}	75.0^{c}	180.0^{b}	100.0^{c}	140.0^{c}
24 July	33.3^{c}	63.3^{c}	48.3^{c}	80.0^{c}	70.0^{c}	75.0^{d}
Mean	279.7^{a}	158.1^{b}		311.8^{a}	195.2^{b}	
SE±						
Variety		23.0			26.5	
Sowing date		39.8			43.3	
Interaction		56.2			59.7	
CV%		31.5			28.8	

Means with the same letter(s) in the same column are not significantly different at the 5% level of significance.

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Short communication

CROP-WEED COMPETITION STUDIES IN FABA BEAN (*Vicia faba* L.) UNDER RAINFED CONDITIONS

A. M. TAWAHA and M. A. TURK

DEPARTMENT OF PLANT PRODUCTION, FACULTY OF AGRICULTURE, JORDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, IRBID, JORDAN

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Field experiments were conducted during the two growing seasons of 1999 and 2000 at the research farm of the Jordan University of Science and Technology (JUST) to study the effect of the time of weed removal on the yield and yield components of faba bean (*Vicia faba* L.). Removing weeds from 25 to 75 days after crop sowing led to significantly larger yields than on plots which were not weeded. Maximum yield was obtained in both years when weeds were removed thrice at 25, 50 and 75 days after crop sowing.

Key words: *Vicia faba* L., hand weeding, weed competition, rainfed conditions, *Brassica nigra* L.

Introduction

Faba bean (*Vicia faba* L.) is the fourth most important pulse crop in the world. It occupies the greatest area planted to legume crops in the Arab countries (Amin, 1988). Faba bean is a valuable food legume rich in proteins and carbohydrate (Karamanos et al., 1994). In Jordan faba bean is primarily consumed as fresh pods and secondarily used for dry seed production. It is grown as a winter crop under both rainfed and irrigated conditions, mainly in the Jordan valley. During 1995 the total production of dry seeds of faba bean under rainfed conditions averaged 700 tons, cultivated on an area of 800 ha (Anonymous, 1995). In general, the total production of faba bean in Jordan is low and far below the country's needs. The average seed yield in Jordan was 0.93 tons ha^{-1} during the period 1990–1995 (Anonymous, 1995). As a result, high quantities of seed were imported. In 1992, approximately 8600 tons were imported, which cost the country 3.32 million U.S.$ (AOAD, 1993). Nevertheless, Dantuma and Thompson (1983) indicated that carefully husbanded crops of faba bean could yield more than 6 tons ha^{-1} of dry seed and provide satisfactory profits for the grower. The low yield obtained locally may be attributed to several factors, including low and erratic rainfall, the planting of traditional low-yielding cultivars and poor cultural practices, such as lack of effective weed control measures and basic knowledge of weed management (Duwayri et al., 1988). Weeds compete for water, light, nutrients and space, and their control is the most difficult and costly item in crop production. Growers often assume, erroneously, that removing weeds at any time during the growing season is equally beneficial to the crop. However, substantial evidence (Zimdahl, 1980, in ground nut; Yadav and Singh, 1984, in groundnut) indicates that the time of removal is as important as removal *per se*. The present study investigated the influence of weed removal at different times after sowing on the crop growth and yield of faba bean.

Materials and methods

Field experiments were conducted at the research farm of the university in Houfa, northern Jordan during the two growing seasons of 1999 and 2000. The location has a Mediterranean climate with mild rainy winters and dry hot summers. The soil is very fine montmorillonitic Thermic Entic Chromoxererts. The soil pH is 7.9. A fertilizer treatment of 20 kg ha^{-1} of P_2O_5 was applied and mixed with the soil prior to planting. The experiments were laid out in a randomized block design with three replicates. The plots were 2.5 m long and 2.4 m in width. Each plot contained 6 rows and the spacing between and within rows was 0.4 m and 0.1 m, respectively, resulting in a planting density of about 25 plants m^{-2}. The faba bean cultivar cv. Major was sown on December 1st and November 29th in the 1999 and 2000 growing seasons, respectively. Harvesting was done on May 18th 1999 and June 10th 2000. The experiment consisted of 12 treatments: Unweeded treatment, removal of weeds once at 25, 50, 75 or 100 days after sowing (DAS), twice at 25 and 50, 25 and 75 or 25 and 100 DAS, thrice at 25, 50 and 75, 25, 75 and 100 or 50, 75 and 100 DAS, and a weed-free treatment. The intensity of weed incidence was recorded using a quadrate of 50 × 50 cm placed on each plot. The measured variables included seed yield (kg ha^{-1}), 100 seed weight (g), pods $plant^{-1}$, seeds pod^{-1}, branches $plant^{-1}$, plant height (cm), flowers $plant^{-1}$, 50% flowering, physiological maturity, number and weight of weeds m^{-2}. The analysis of variance and LSD mean separation were performed using the computer statistical program MSTAT-C for a randomized complete block design as described by Steel and Torrie (1980). Comparisons between means were made using the least significant difference test (LSD) at the 0.05 probability level.

Results and discussion

Treatment effects on weeds

The data given in Tables 1 and 2 reveal that all the weeding treatments significantly reduced the weed intensity and weed dry matter as compared to the unweeded control in both years. The weed flora of the experimental field consisted of *Hordeum vulgare* L., *Vaccaria pyramidata* L., *Brassica nigra* L., *Convolvulus arvensis* L., *Molucella laevis* L., *Galium tricornutum*, *Anemone coronaria* L. and *Tetragonolobus palaestinus* Boiss. There were more weeds present in the 2000 growing season than in 1999. The maximum number of weeds was recorded in the treatment with the removal once at 100 days after crop sowing. Removing weeds once at 25, at 50, or at 75 days after sowing (DAS), removing weeds twice at 25 and 75 DAS or at 50 and 75 and removing weeds thrice at 25, 50 and 75 DAS led to successively fewer weeds being present at 100 days after crop sowing than in the unweeded treatment (Table 1). The dry weight of weeds followed similar trends to the number of weeds (Table 2). All weeding treatments led to significantly less weed dry weight at 100 days after crop sowing than in the unweeded plots. Weed removal at later stages of crop development gave the least dry weight of weeds at 100 days after crop sowing, since few additional weeds had time to emerge.

Table 1

Number of weed plants/m^2 present at 100 days after sowing the crop

Treatments	*Convolvulus arvensis* L.		*Molucella laevis* L.		*Brassica nigra* L.		*Galium tricornutum*		*Vicia narbonensis* L.		Total	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Weed free	0	2	5	7	2	3	4	5	0	0	11d	17f
Unweeded check	1	3	7	7	38	38	7	9	18	16	71a	73a
Weeding once at 25 DAS	2	3	6	8	7	9	8	10	8	11	31b	41b
Weeding once at 50 DAS	1	3	9	12	6	5	8	10	8	10	32b	40b
Weeding once at 75 DAS	1	2	18	18	4	4	4	6	0	2	27b	32cd
Weeding once at 100 DAS	1	3	7	7	38	36	7	9	18	20	72a	75a
Weeding twice at 25+50 DAS	1	3	10	12	6	3	8	10	2	3	27b	31cd
Weeding twice at 25+75 DAS	1	2	10	12	4	4	4	4	0	3	19c	25de
Weeding twice at 25+100 DAS	11	3	10	11	7	9	5	5	8	10	31b	38bc
Weeding thrice at 25+50+75 DAS	0	2	2	4	4	6	4	3	5	7	15cd	22ef
Weeding thrice at 25+75+100 DAS	1	2	2	4	4	6	4	6	0	0	11d	18ef
Weeding thrice at 50+75+100 DAS	1	2	3	4	4	7	4	3	0	*2*	13cd	18ef
LSD (0.05)											7.8	7.9

Means in each column followed by the same letters are not significantly different according to LSD (P 0.05).

Table 2

Weight of weed dry matter (g/m^2) removed at 100 days after sowing the crop

Treatments	*Convolvulus arvensis* L.		*Molucella laevis* L.		*Brassica nigra* L.		*Galium tricornutum*		*Vicia narbonensis* L.		Total	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Weed free	0	0.28	0.90	1.26	3.4	4.8	1.00	1.00	0	0	5.3f	7.34f
Unweeded check	0.15	0.35	1.26	1.18	72.2	68.4	1.75	2.00	6.70	8.60	82.1a	81.5a
Weeding once at 25 DAS	0.20	0.33	1.00	1.30	11.2	16.2	1.70	2.10	2.90	4.10	17.0c	24.0b
Weeding once at 50 DAS	0.18	0.29	1.50	2.00	10.8	9.5	1.60	2.00	2.80	3.60	16.9c	17.4cd
Weeding once at 75 DAS	0.17	0.28	3.00	2.90	6.0	7.2	1.00	1.30	0	0.70	10.2de	12.4def
Weeding once at 100 DAS	0.24	0.35	1.30	1.30	5.7	68.4	1.75	2.25	6.30	7.00	67.8b	79.3a
Weeding twice at 25+50 DAS	0.18	0.40	1.80	2.20	6.0	5.6	1.80	1.60	0.64	1.10	10.4de	10.9ef
Weeding twice at 25+75 DAS	0.18	0.36	1.60	2.00	6.0	7.6	1.25	1.30	0	1.10	9.0ef	10.6ef
Weeding twice at 25+100 DAS	0	0.28	1.70	2.00	11.2	15.3	1.10	0.72	2.80	2.90	16.8c	21.2cd
Weeding thrice at 25+50+75 DAS	0.18	0.36	0.36	0.50	11.0	14.0	1.00	1.30	1.80	2.20	14.3cd	18.4c
Weeding thrice at 25+75+100 DAS	0.13	0.28	0.44	0.49	8.0	13.0	1.00	0.75	0	0	9.6ef	14.5cde
Weeding thrice at 50+75+100 DAS	0.13	0.25	0.49	0.45	7.5	11.0	1.20	0.50	0	0.38	9.3ef	12.6def
LSD (0.05)											4.5	

Means in each column followed by the same letters are not significantly different according to LSD (P 0.05).

Table 3

Effect of time of weed removal on seed yield, plant height, 100-seed weight, seeds pod^{-1} and primary branches $plant^{-1}$ of faba bean

Treatments	Seed yield kg ha^{-1}		Plant height (cm)		100 seed weight (g)		Seeds pod^{-1}		Primary branches $plant^{-1}$	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Weed free	1620a	1800a	85a	93a	80a	90a	3a	3a	3a	4a
Unweeded check	1250e	1300f	55f	59g	70c	75de	3a	3a	1c	1.7b
Weeding once at 25 DAS	1400bc	1650cd	70c	76d	78ab	81bc	3a	3a	3a	4a
Weeding once at 50 DAS	1300de	1550e	60e	63f	74bc	78cd	3a	3a	2b	2.3b
Weeding once at 75 DAS	1300de	1550a	60e	63f	74bc	77cd	3a	3a	1c	1.7b
Weeding once at 100 DAS	1250e	1300f	55f	59g	70ab	75e	3a	3a	1c	1.7b
Weeding twice at 25+50 DAS	1540a	1690bc	70c	76d	78b	82bc	3a	3a	3a	4a
Weeding twice at 25+75 DAS	1350cd	1600de	65d	70e	76b	79bcd	3a	3a	3a	4a
Weeding twice at 25+100 DAS	1370bcd	1600de	65d	70e	75ab	79bcd	3a	3a	3a	4a
Weeding thrice at 25+50+75 DAS	1550a	1750ab	80b	87b	78ab	90a	3a	3a	3a	3.7a
Weeding thrice at 25+75+100 DAS	1450b	1700bc	74c	82c	78ab	84b	3a	3a	3a	4a
Weeding thrice at 50+75+100 DAS	1400bc	1683bc	70c	76d	78ab	82bc	3a	3a	2b	2.3b
LSD (0.05)	43	35	8.1	6.8	4.3	5.1	NS	NS	0.17	0.7

Means in each column followed by the same letters are not significantly different according to LSD ($P \leq 0.05$).

Table 4

Effect of time of weed removal on pods $plant^{-1}$, flowers $plant^{-1}$, days to 50% flowering and days to physiological maturity of faba bean

Treatments	Pods $plant^{-1}$		Flowers $plant^{-1}$		Days to 50% flowering		Days to physiological maturity	
	1999	2000	1999	2000	1999	2000	1999	2000
Weed free	8a	11a	38a	43a	93e	96c	158a	162a
Unweeded check	4d	4f	13f	18fg	99a	101a	151e	156d
Weeding once at 25 DAS	6b	8b	18d	21e	94d	98b	154bc	157c
Weeding once at 50 DAS	5c	8b	18d	22de	96c	98b	155c	157c
Weeding once at 75 DAS	4d	5e	15e	19f	96c	98b	155c	157c
Weeding once at 100 DAS	6b	4.3f	13f	15g	99a	101a	152e	153g
Weeding twice at 25+50 DAS	6b	8b	20b	24bc	94d	98b	156b	155e
Weeding twice at 25+75 DAS	6b	8b	19c	24bc	94d	98b	156b	156d
Weeding twice at 25+100 DAS	6b	6d	18d	23cd	94d	94d	154d	154f
Weeding thrice at 25+50+75 DAS	8a	11a	38a	42a	93e	93e	159a	156d
Weeding thrice at 25+75+100 DAS	6b	7c	20b	25b	94d	94d	156b	160b
Weeding thrice at 50+75+100 DAS	6b	8b	18d	23cd	94d	94d	155c	157c
LSD (0.05)	0.97	0.62	0.9	1.3	0.9	1.2	0.93	0.99

Means in each column followed by the same letters are not significantly different according to LSD ($P \leq 0.05$).

Treatment effects on yield, yield components and phenological traits of faba bean

In both growing seasons, seed yield, 100-seed weight, pods plant^{-1}, primary branches plant^{-1}, plant height and flowers plant^{-1} were greatest in the clean-weeded treatment and least in the unweeded control (Tables 3 and 4). If weeds were not controlled until 100 days after sowing, they reduced the seed yield by 125 and 130 kg ha^{-1} in 1999 and 2000, respectively. The number of seeds per pod was not affected by the weed treatments. The optimum time for weeding was thrice at 25, 50 and 75 days after crop sowing: the yield did not differ greatly from the clean-weeded treatment. Weeding once at 25 days after crop sowing gave less yield than the weed-free plots. There was a steady decline in yield with later weeding. The data presented in Table 4 describe the effect of the time of weed removal on 50% flowering and physiological maturity (number of days from the sowing date) during the two growing seasons of 1999 and 2000. During both growing seasons, statistical analyses indicated significant differences between the different hand weeding treatments in the time required to reach these stages. A prolongation of the period required to reach each of the phenological traits (Table 4) was associated with the unweeded control. The critical period of crop weed competition was from 25 to 75 days after crop sowing. Weeding thrice at 25, 50 and 75 days after crop sowing prevented weed competition (for light, nutrients, moisture and space) during this critical crop period and in turn resulted in better yield and yield components.

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Short communication

PERFORMANCE OF VEGETABLE COWPEA (*Vigna unguiculata* L. Walp) AS INFLUENCED BY P FERTILIZER IN S.E. NIGERIA

B. F. D. OKO, A. E. ENEJI[1], E. EREMI, C. NWOKO and J. O. SHIYAM

DEPARTMENT OF CROP SCIENCE, UNIVERSITY OF CALABAR, CALABAR, NIGERIA
[1]FACULTY OF AGRICULTUTRE, TOTTORI UNIVERSITY, JAPAN

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Four cultivars of cowpea (IT 86F-2089-5, IT 81D-1228-14, IT 92KD-267-2 and IT 92KD-266-2-1) were subjected to four levels of phosphorus fertilizer in Calabar, southeastern Nigeria during the 1995 cropping season. Morphological differences were minimal between the erect and prostrate, bushy types. Significant cultivar differences for green pod yields were obtained, with IT 86F-2089-5 giving the highest and IT 92KD-266-2-1 the lowest yields. For all cultivars except IT 81D-1228-14, the phosphorus fertilizer effect increased with increasing rate of application. The green pod yields of IT 86F-2089-5 and IT 81D-1228-14 were superior to those of other cultivars, whereas the grain yield was highest in the latter cultivar. In addition, IT 81D-1228-14 gave high yields even without P fertilizer, hence it could be a useful variety for low-input farmers in the area.

Key words: phosphorus fertilizer, vegetable cowpea, pod yield

Introduction

Cowpea is an African crop grown throughout the tropics and used as a pulse, vegetable, fodder and cover crop. As a vegetable, the crop is commonly a supplement in the meals of most traditional farmers and is highly favoured by foreign and enlightened urban dwellers.

The present day cultivation of cowpea in Nigeria centres mainly on the production of dry grains. Over 90% of the crop is grown in the savanna region of Northern Nigeria. The cultivation of grain cowpea is not popular in the humid south of Nigeria due to low productivity and lack of improved seeds. However, farmers in this area could and do grow vegetable cowpea as well as other leguminous crops in both the regular and late farming periods. Because of the lack of adequate fertilizers in Nigeria, efforts are being made by researchers to develop cowpea varieties with enhanced nitrogen fixation, efficient use of phosphorus and tolerance to aluminium toxicity, so that cowpea production can be increased in marginal soils. Furthermore, the high price of vegetable cowpea, especially during the off-season, has made it necessary to evaluate some vegetable cowpea cultivars already developed by the International Institute of Tropical Agriculture (IITA), Ibadan in different agro-ecological zones in Nigeria.

The production of vegetable cowpea under conventional and intensive cropping systems will to some extent depend on the use of appropriate fertilizers. Summerfield et al. (1974) reported that the nutrient requirements of cowpea were similar to other crops except in their potential for the symbiotic assimilation of atmospheric nitrogen, which reduces the demand for mineral nitrogen and creates special demands for some micronutrients and phosphate. The low yield under traditional farming systems is partly due to its being grown in association with cereals like sorghum, millet or maize (Summerfield et al., 1974; Rachie and Roberts, 1974). As a monocrop, cowpea responds well to nutrient (N, P and K) application and high yields can be achieved particularly with the use of improved cultivars. Among the nutrients, P is critical in influencing cowpea nodulation and yields in various soils (Aggrey, 1973; Osiname, 1978; Swami and Pal, 1974; Rachie and Roberts, 1974). Kang and Nangju (1983) noted that P application influenced the content of other nutrients in the leaves and Omueti and Oyenuga (1970) reported that the presence of other nutrients in the seed was influenced by P application.

Most farmland holdings in urban Calabar have been continually cropped for several years and need to be carefully managed for sustainable crop production, including cowpea. The site chosen for this trial is classified as low fertility soil and is especially deficient in phosphorus. The need for appropriate fertilization in evaluating the four cowpea cultivars chosen for this trial necessitated the present study.

Materials and methods

The trial was carried out at the Teaching and Research Farm of the University of Calabar (8° 27'E; 4° 58'N), Nigeria. Total annual rainfall in the area is about 2000 mm, while temperatures range between 23 and 33°C. The relative humidity is about 86%. Before the commencement of the present investigation, the plot had been cultivated with cassava (*Manihot esculenta*) continuously for 3 years without fertilizer use. The soil (Crest Typic Tropudalf or Orthic Luvisol) at the experimental site had the following characteristics: pH(H_2O) 4.5, organic carbon (as determined by the method of Walkley and Black, 1934) 32 g kg^{-1}, total N (according to the method of Jackson, 1962) 1.5 g kg^{-1}, exchangeable K (determined from an extract of ammonium acetate with a flame photometer) 0.14 cmol kg^{-1} and available P (Bray–1, Bray and Kurtz, 1945) 1.6 mg kg^{-1}.

Four bushy types of vegetable cowpea cultivars, IT 86F-2089-5, IT 81D-1228-14, IT 92KD-267-2 and IT 92KD-266-2-1 from IITA were planted in sixty-four conventionally tilled plots, each measuring 3 m × 3 m. The plots were separated from each other by a 1 m alleyway. The treatments were laid out in a 4 × 4 factorial in randomised complete blocks with 3 replications. The four cowpea varieties constituted the main plots while four rates of phosphorus application (0, 20, 40 and 60 kg P_2O_5 ha^{-1}) constituted the subplots. A basal dose of 30 kg N ha^{-1} was applied to provide initial N for the plants.

Three seeds were sown per hill at a spacing of 1 m × 0.2 m, and later thinned to one seedling per hill, giving a population of 50,000 plants ha^{-1}. The plots were hand-weeded twice before the cowpea was harvested. Crop evaluations were carried out by both visual observations (e.g. morphological characteristics) and measurement of parameters of interest such as pod and grain yield. The green pod harvest commenced some 49–50 days after sowing and pods that were too mature to harvest green were harvested at physiological maturity. All other pods not harvested green were allowed to dry out in the field. Final pod yields were obtained from the cumulative yields for each variety. The data were analysed statistically using analysis of variance and the means were separated by Duncan's multiple range test.

Results and discussion

The morphological characteristics of the four cowpea cultivars are presented in Table 1. Morphological differences were minimal between the erect and prostrate bushy types. Seedling emergence, seedling to maturity duration, immature pod colour, pod shape, seed shape and percentage shattering were generally uniform among the cultivars. Slight variations in plant height, number of branches, colour of flowers, pod length, number of seeds per pod, seed and eye colour were observed.

The pod length, which has implications for yield, was 27–30 cm for IT 81D-1228-14 and IT 86F-2089-5, 16–18 cm for IT 92KD-266-2-1 and 15–18 cm for IT 92KD-267-2. The pod length was shorter for the prostrate types, which also had lower seed number per pod: 9–14 for IT 92KD-266-2-1 and 10–14 for IT 92KD-267-2 as compared to the erect types with 14–16 seeds per pod for IT 81D-1228-14 and 16–18 seeds per pod for IT 86F-2089-5. IT 92KD-2662-2-1, however, had a greater number of branches than any other cultivar. Preference should therefore be given to cultivars that tend to have large yield potentials, such as IT 86F-2089-5 for seed number per pod and IT 92KD-266-2-1 for profuse branches. IT 86F-2089-5 has an added advantage due to its longer pod length, which is usually more appealing to consumers.

The effects of P on the green pod yield of the vegetable cowpeas are presented in Table 2. Averaged across P levels, the pod yields were in the order: IT 86F-2089-5 (1414 kg ha^{-1}) > IT 81D-1228-14 (862 kg ha^{-1}) > IT 92KD-267-2 (807 kg ha^{-1}) > IT 92KD-266-2-1 (508 kg ha^{-1}). The performance of IT 86F–2089–5 appears to corroborate the observations on the morphological potential of the cultivar. The profuse branching potential of IT 92KD-266-2-1 failed to confirm expectations that this trait would result in greater yields arising from the expected larger pod number. IT 81D-1228-14 performed remarkably well even without P application, and at 20 kg P ha^{-1} it gave the highest pod yield. At 60 kg P ha^{-1}, IT 86F-2089-5 gave the best pod yield. In a study in Burkina Faso, Muleba and Coulibaly (1999) reported that the optimum rate of single super phosphate for cowpea was 21.8 kg ha^{-1}. The critical P limit for corn and cowpea was studied on an Oxisol in the Amazon region of Brazil (Smyth and Cravo, 1990). On low P status soils, significant responses were obtained in earlier studies (Tewari, 1965; Aggrey, 1973; Osiname, 1978; Swami and Pal, 1974; Rachie and Roberts, 1974). In the present study, the marked differences in cultivar yields could also have been due to Al and Mn toxicity, which is associated with the low pH soils in the area. Further studies are needed to verify this hypothesis, since plant tissue Al and Mn concentrations were not determined during the present investigation.

Across the varieties, except IT 81D-1228-14, the green pod yields increased with increasing P rates. The interaction between cowpea cultivar and P levels was not significant in this study. For IT 86F-2089-5, the green pod yield ranged from 592 kg ha^{-1} in the control to 2025 kg ha^{-1} at 60 kg P ha^{-1}, while that of IT 81D-1228-14 ranged from 822 kg ha^{-1} in the control to 1069 kg ha^{-1} at 20 kg P ha^{-1}. Economic considerations dictate that yields exceeding one metric ton ha^{-1} for these cultivars at any given level of P should be preferred, since such yields would attract greater economic returns.

Table 1

Some morphological and other features of four vegetable cowpea cultivars

Plant features	IT 81D-1228-14	IT 86F-2089-5	IT 92KD-266-2-1	IT 92KD-267-2
Emergence (%)	60	54	58	53
Growth habit	Erect & bushy	Erect & bushy	Prostrate & bushy	Prostrate & bushy
Plant height (cm)	50–80	60–70	75–85	75–85
No. of branches	5–6	4–6	15–18	4–5
Flower colour	Purple	Light orange to purple	White	White
Pod length (cm)	27–30	27–30	16–18	15–18
No. of seeds/pod	14–16	16–18	9–14	10–14
Seed colour	White	Mottled	Mottled	White
Eye colour	White/black ring	White/black ring	White/black ring	Brown/black ring

Growth duration, days to maturity, pod shape and seed shape were similar for all the cultivars

The phosphorus effect on grain yield (Table 2) showed the following order: IT 81D-1228-14 (135 kg ha^{-1}) > IT 92KD-267-2 (124 kg ha^{-1}) > IT 86F-2089-5 (95 kg ha^{-1}) > IT 92KD-266-2-1 (78 kg ha^{-1}). The grain yields were lower than usual because these are vegetable cultivars grown only for the green pods. The grains only complement the target green pod yields. From the results of green pod and grain yields, IT 86F-2089-5 and IT 81D-1228-14, both erect bushy types, would be preferred to IT 92KD-266-2-1 and IT 92KD-267-2 for the Calabar environment. IT 81D-1228-14 was able to give high green pod yields even without P application. In addition, this cultivar consistently gave the best pod and grain yields at the low application rate of 20 kg P ha^{-1}. Therefore, for low-input farmers in the study area, IT 81D-1228-14 is recommended.

Table 2

Phosphorus effect on green pod and grain yields (kg/ha) of four cowpea cultivars

Cultivar	Phosphorus levels (kg/ha)								Cultivar mean	
	0		20		40		60			
	A	B	A	B	A	B	A	B	A	B
IT 86F-2089-5	591.7b	122.2a	1194.4a	72.2b	1845.8a	86.4b	2025.0a	97.2b	1414.2a	94.5b
IT 92KD-267-2	433.3c	144.7a	847.2b	123.8a	958.5b	116.7ab	990.3b	108.3ab	807.3b	124.4a
IT 81D-1228-14	822.2a	133.3a	1069.4a	144.5a	797.5bc	130.6a	758.3c	130.6a	861.9b	134.7a
IT 92KD-266-2-1	394.4c	58.3b	502.7c	80.6b	513.5c	91.7b	622.1d	80.6b	508.2c	77.8b
Phosphorus mean	560.4	114.7	903.5	106.3	1028.8	106.3	1098.9	104.2		

Means in the same column followed by the same letter are not significantly different at $p = 0.05$.
A = green pod yield; B = grain yield.

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Manuscripts and editorial correspondence should be addressed to

Acta Agronomica Hungarica
Agricultural Research Institute of the
Hungarian Academy of Sciences
H-2462 Martonvásár, Hungary
Phone: (36-22) 569-521
Fax: (36-22) 460-213
E-mail: actaagr@mail.mgki.hu

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ORIGINAL PAPERS

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INFLUENCE OF TI(IV)-ASCORBATE ON SOLUBLE CARBOHYDRATE CONTENT IN WHEAT SEEDLINGS EXPOSED TO CADMIUM

I. KEREPESI[1], É. STEFANOVITS-BÁNYAI[2], J. KISPÁL[1] and É. SÁRDI[3]

[1]DEPARTMENT OF GENETIC AND MOLECULAR BIOLOGY, UNIVERSITY OF PÉCS, PÉCS, HUNGARY
[2]DEPARTMENT OF APPLIED CHEMISTRY, FACULTY OF FOOD SCIENCE, SZENT ISTVÁN UNIVERSITY, BUDAPEST, HUNGARY
[3]DEPARTMENT OF MOLECULAR PLANT BIOLOGY, FACULTY OF HORTICULTURAL SCIENCE, SZENT ISTVÁN UNIVERSITY, BUDAPEST, HUNGARY

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The water-soluble carbohydrates contributing to the response of wheat seedlings to cadmium stress in nutrient solution were studied with or without Ti(IV)-ascorbate supply. The total water-soluble carbohydrate, glucose, fructose, sucrose, glucan and fructan contents, and the cadmium and titanium contents were measured in wheat seedlings exposed to 10^{-4} M Cd or 10^{-5} M Cd with either Ti(IV)-ascorbate or Na-ascorbate in the medium.

Glucose, fructose and fructan showed the greatest response to Cd, ascorbate and titanium treatments. The sugar content in plants exposed to Cd increased with the metal concentration. Titanium tended to decrease the cadmium-induced sugar accumulation.

Ti(IV)-ascorbate and Na-ascorbate were also applied without Cd to study the effect of these chemicals. In general, Na-ascorbate induced a higher accumulation of sugar components than Ti(IV)-ascorbate.

Titanium addition in Cd-containing solution caused a significant decrease in the cadmium accumulation in the leaves. An increase in titanium content was observed only in the roots, higher values being measured in plants grown in solution containing 10^{-4} M Cd.

Key words: cadmium, carbohydrates, titanium, wheat

Abbreviations: WSC, total water-soluble carbohydrate

Introduction

Cadmium is known to be one of the most toxic metals released in the environment. Elevated levels of cadmium may cause severe phytotoxic effects characterised by changes in plant growth, photosynthesis and transpiration (Kabata-Pendias and Pendias, 1985).

The fundamental processes of carbohydrate production and utilisation in green plants are controlled by a number of key factors. Several studies, mostly in the laboratory, indicate an altered carbohydrate and nitrogen metabolism in plants affected by metal exposure. In these studies the concentrations of sugars, starch and certain amino acids are usually elevated (Lamoreaux and Chaney, 1978). In parallel the effect of Ti(IV) on nutrient uptake and plant metabolism was investigated. The increased absorption of all nutrients was observed in the presence of titanium in a large variety of crops (Feher et al., 1987; Kiekens and Camerlynck, 1987; Pais, 1983). Studies on *Capsicum annuum* showed that the concentrations of some essential macro- and microelements were enhanced when plants were supplied with Ti(IV) (Giménez et al., 1990). The fact that this increase was greater under insufficient nutrient supplies suggests a positive effect of titanium in moderating stress effects. In other experiments N (Frutos et al., 1996) and P (Lopez-Moreno et al., 1996) were partially withdrawn from the fertiliser applied to Ti-treated crops and this reduction did not cause any nutritional imbalance in the plants. Numerous reports suggest the importance of titanium in metabolic processes. Increases in the catalase, peroxidase and nitrate reductase activity were observed after the addition of Ti to *Triticum aestivum* (Pais, 1983), while decreases in the phosphofructokinase activity (Simon et al., 1990) and starch content (Carvajal and Alcaraz, 1998; Pais, 1983) were recorded.

This study aimed to examine the influence of Ti(IV)-ascorbate on the soluble carbohydrate content in wheat seedlings exposed to cadmium. More precisely, the objectives were (1) to assess and characterise the involvement of WSC and the main sugar components in these cadmium- and/or Ti-induced processes and (2) to determine the role of ascorbate in this effect. The hypothesis was that titanium reduces the toxic effect caused by cadmium in the nutrient solution and that this behaviour of titanium was correlated with changes in the soluble carbohydrate content. Therefore, hydroponically grown wheat seedlings were used to measure changes in soluble carbohydrate contents induced by cadmium and titanium exposure as well as changes in the concentration of titanium and cadmium in leaves and roots.

Materials and methods

Plant material: winter wheat (*Triticum aestivum* L. cv. Martonvásári 21).

The seeds were grown hydroponically in complete nutrient solution in a growth chamber, with a 16 h daylight period at 23°C, and an 8 h dark period at 18°C. From the 7th to the 18th day the plants were exposed to Cd and Ti(IV)-ascorbate stresses in the following combinations: Cd 10^{-4} M; Cd 10^{-5} M; Cd 10^{-4} M plus Ti(IV)-ascorbate (5 μg/l); Cd 10^{-5} M plus Ti(IV)-ascorbate (5 μg/l); Cd 10^{-4} M plus Na-ascorbate (0.91 M); Cd 10^{-5} M plus Na-ascorbate (0.91 M); Ti(IV)-ascorbate (5 μg/l); Na-ascorbate (0.91 M). The plants were sampled on the 3rd, 7th and 11th days after the treatments and separated into leaves and roots. Processing and extraction were started immediately. The present paper deals mostly with the leaf data.

The extraction of the samples and the chemical analyses of the carbohydrates were carried out according to Kerepesi et al. (1996). The cadmium and titanium contents were determined with a Jarrell-Ash polychromator ICP spectrometer: 0.2 g dry material was treated in a mixture of 2 cm^3 concentrated HNO_3 and 2 cm^3 concentrated H_2O_2 overnight, digested for 20–25 minutes and diluted with 2 M HNO_3 to 10 cm^3. Three replications of each experiment were performed.

Results and discussion

In agreement with earlier reports the results show that water-soluble carbohydrate contents are a very sensitive trait for indicating environmental effects (Housley and Pollock, 1993; Kerepesi et al., 1998). Although several papers have indicated a Ti(IV)-ascorbate-mediated positive effect on the metabolism in *Triticum aestivum* (Pais, 1983; Carvajal and Alcaraz, 1998), this is the first report which presents data on the influence of Ti(IV)-ascorbate and ascorbic acid on the main soluble carbohydrate components in wheat exposed to cadmium in nutrient solution.

The plants responded to 10^{-4} M Cd with an increase in WSC content, while those treated with 10^{-5} M Cd only exceeded the control value at day 7 (Table 1). Plants exposed to 10^{-4} M Cd accumulated significantly ($P<0.05$) more sugar than those exposed to 10^{-5} M Cd.

The differences detected in cadmium-induced WSC were also related to the presence of Na-ascorbate or Ti(IV)-ascorbate in the nutrient solution. From day 7, the Cd plus Na-ascorbate treatment induced higher sugar accumulation at both Cd concentrations compared with the control (Table 1). Ti-ascorbate behaved differently from Na-ascorbate, resulting in a significantly ($P<0.001$) lower sugar content: the WSC content in Cd plus Ti(IV)-ascorbate treated plants was similar to or lower than the control.

Ti(IV)-ascorbate and its component ascorbic acid were also applied without Cd, to study the effect of these chemicals alone (Table 1). WSC increased markedly after Na-ascorbate treatment at days 3 and 7 ($P<0.05$), while by day 11 the WSC content was similar to the control. Plants treated with Ti(IV)-ascorbate showed similar or lower (3rd day) WSC contents compared with the control plants.

Considering the components of the total soluble carbohydrates, changes in the responses of the individual components were evident. Usually, the sugar content in plants exposed to Cd increased and this increment was higher in the case of higher metal concentration. Furthermore, Ti(IV)-ascorbate tended to decrease the cadmium-induced sugar accumulation.

Glucose, fructose and fructan showed the greatest response to Cd, Ti(IV)-ascorbate and Na-ascorbate treatments (Fig. 1). Glucose and fructose accumulation (at Cd 10^{-4} M) was the earliest response, followed by fructan and/or glucan. These observations are in agreement with the report of Munns and Weir (1981), who reported that the initial changes in osmotic potential were largely due to reducing sugars.

There was a significant ($P<0.01$) increase in the glucose content by day 3 in plants exposed to Cd 10^{-4} M and to the Cd (both concentrations) plus Na-ascorbate treatment. The Cd plus Na-ascorbate treatment caused higher glucose accumulation than the Cd treatment. The Cd plus titanium treatment resulted in a decrease (0.5 fold) in the glucose level at both Cd concentrations on the 3rd day, but these differences disappeared completely by day 11. Fructose showed similar behaviour, but the differences remained during the whole treatment. In contrast to these results, Malic et al. (1995) found that the concentrations of various metabolites, including glucose, fructose and sucrose, decreased in 30-day-old wheat seedlings treated with 10 mM and 20 mM cadmium. Greger et al. (1991) reported an increasing sugar level in 14-day-old sugar beet exposed to 0.6–20 mM cadmium in daily increments. In the present experiments cadmium stress increased the soluble carbohydrate content, and higher cadmium concentration caused a higher rate of accumulation. One explanation is that the decrease in the utilisation of carbohydrate for growth is more pronounced than the decrease in CO_2 fixation, thus resulting in an increased accumulation of carbohydrates.

Table 1

Changes in total water-soluble carbohydrate content (means±SD mg/g FW) in wheat seedlings in Cd^{++}-containing nutrient solution with the addition of either Ti(IV)-ascorbate or Na-ascorbate

Treatments	Days		
	3	7	11
Cd^{++} 10^{-4} M	9.29±0.71	5.57±0.46	4.82±0.37
Cd^{++} 10^{-4} M+Asc	8.51±0.76	4.87±0.37	4.54±0.42
Cd^{++} 10^{-4} M+Ti(IV)-Asc	5.88±0.49	4.06±0.35	3.68±0.33
Cd^{++} 10^{-5} M	8.63±0.81	4.12±0.38	2.95±0.13
Cd^{++} 10^{-5} M+Asc	8.39±0.71	4.43±0.31	3.73±0.27
Cd^{++} 10^{5} M+Ti(IV)-Asc	6.63±0.51	3.27±0.27	2.81±0.11
Control	8.12±0.71	3.39±0.25	2.73±0.21
Control+Asc	10.05±0.97	4.17±0.37	2.65±0.19
Control+Ti(IV)-Asc	8.45±0.91	2.70±0.18	2.40±0.21

All the treatments applied caused a higher level of fructan from day 3 compared to the control (Fig. 1). From day 7 the fructan content in Cd-treated plants increased after the addition of Na-ascorbate, while it remained mostly the same after the addition of Ti(IV)-ascorbate. This tendency was observed at both Cd concentrations and throughout the whole experiment. These results were in agreement with findings that the accumulation of fructan is induced by unfavourable conditions. This suggests that, as well as acting as reserve carbohydrates, fructans may have other functions, including involvement in the stress adaptation of many grasses including wheat and barley (Housley and Pollock, 1993; Kerepesi et al., 1998).

A small increment in oligo-glucan content was measured only during 10^{-4} M Cd stress. Ti(IV)-ascorbate and Na-ascorbate caused lower glucan content at 10^{-4} M Cd concentration, but these changes were only observed on day 3.

The data recorded for Na-ascorbate and Ti-ascorbate treated plants showed that these chemicals themselves had an effect on carbohydrate content (Fig. 2). Changes in the individual sugar components showed that in general it was the ascorbate which induced higher accumulation of these components, and not Ti(IV).

Figure 3 shows that the supplementation of Cd-containing solution with titanium caused lower cadmium accumulation in the leaves than was observed without Ti(IV)-ascorbate. At 10^{-5} M Cd concentration the highest cadmium accumulation was measured in Cd+Na-ascorbate treated plants, while at 10^{-4} M Cd the cadmium itself was the most efficient in this aspect. This tendency was manifested to the greatest extent on day 3 with a 1.2-fold higher cadmium accumulation, while the differences were equalised by the end of the study.

Changes in titanium content were only observed in the roots (Table 2), where plants grown in 10^{-4} M Cd-containing solution showed significantly higher values ($P<0.001$). The rate of Ti(IV) accumulation in the roots was about 2.5-fold (Cd 10^{-5} M) and 10-fold (Cd 10^{-4} M) greater than that in the leaves by the end of the experiment. These results confirm the findings of Giménez et al. (1990) that the effect of titanium was stronger at the higher cadmium concentration.

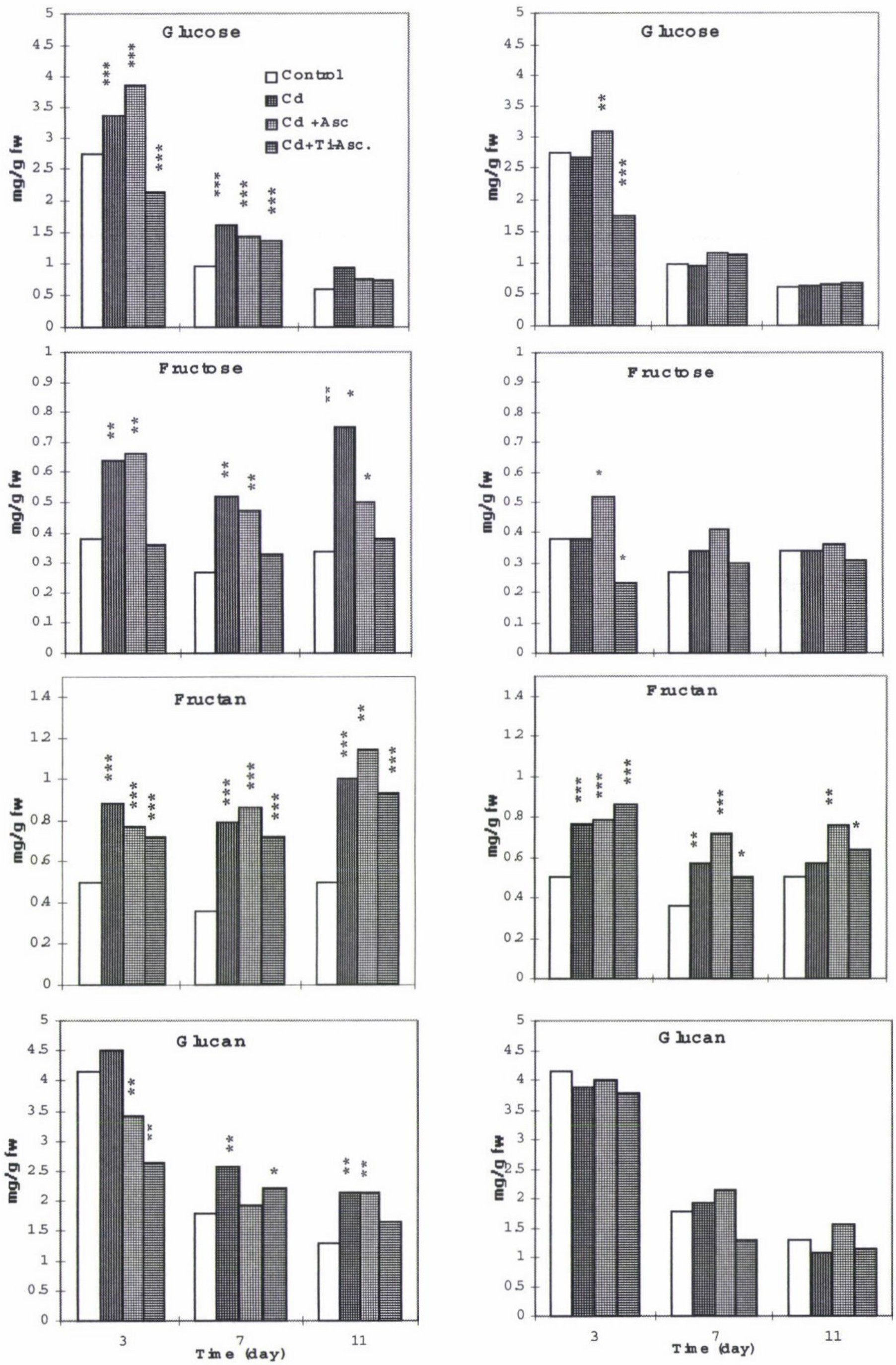

Fig. 1. Carbohydrate content in wheat grown in nutrient solution containing 10^{-4} M or 10^{-5} M cadmium with the addition of either Na-ascorbate or Ti-ascorbate. Differences from the control were significant at the P<0.05 (*), P<0.01 (**) and P<0.001 (***) levels

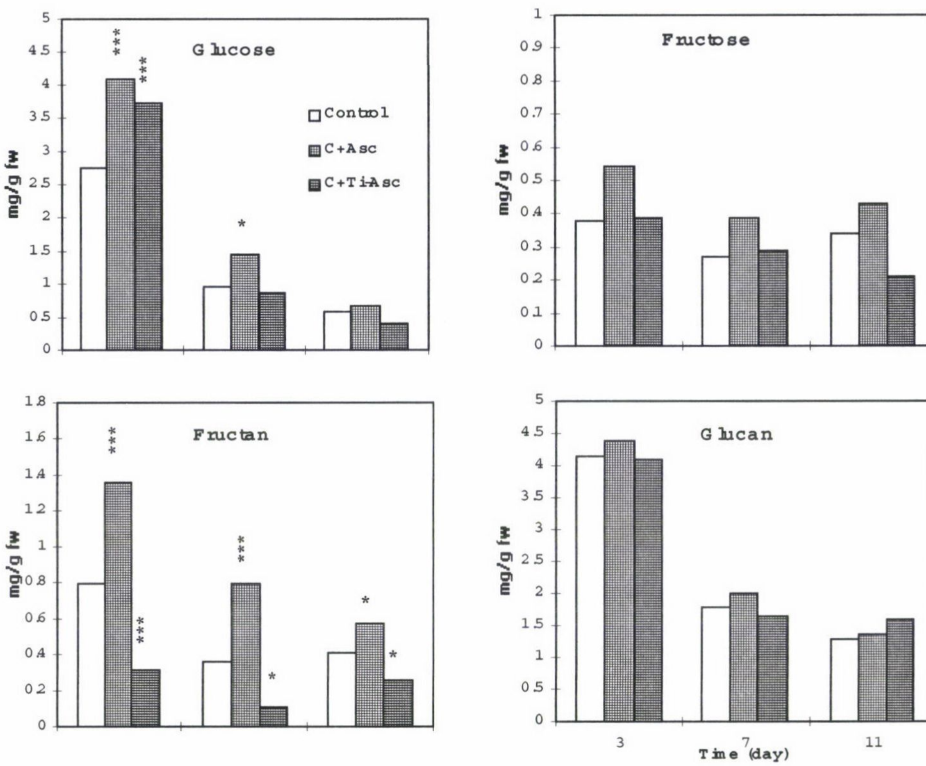

Fig. 2. Carbohydrate content in wheat grown with either Na-ascorbate or Ti-ascorbate in the nutrient solution. Differences from the control were significant at the P<0.05 (*) and P< 0.001 (***) levels.

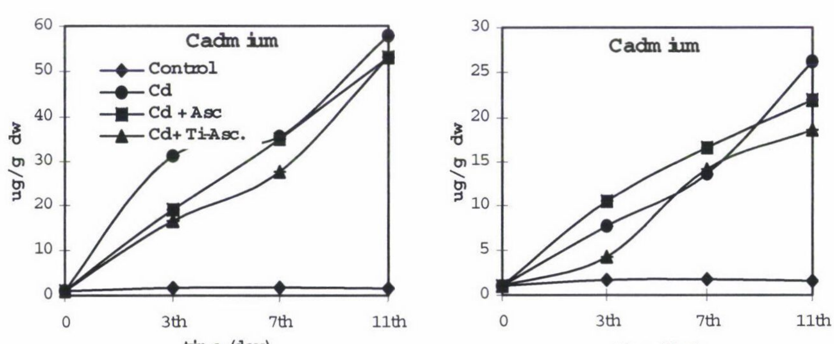

Fig. 3. Cadmium content in the leaves of wheat grown in nutrient solution containing 10^{-4} M or 10^{-5} M cadmium with the addition of either Na-ascorbate or Ti-ascorbate

Table 2
Changes in titanium content (means±SD μg/g DW) in wheat seedlings exposed to Ti(IV)-ascorbate in Cd^{++}-containing nutrient solution

Treatments	Days			Days		
	3	7	11	3	7	11
	Leaf			Root		
Control	1.45±0.50	1.65±0.44	1.85±0.53	0.3±0.09	0.3±0.09	0.47±0.1
Cd 10^{-4} M	1.16±0.41	1.26±0.35	1.85±0.41	5.67±0.98	19.27±4.87	19.02±5.63
Cd 10^{-5} M	1.35±0.52	1.45±0.39	1.76±0.32	3.59±0.72	3.34±0.82	4.02±0.85

Conclusions

The total soluble carbohydrate content could be a useful and very sensitive trait for indicating cadmium-induced metabolic changes in plants. Moreover, monosaccharides and fructans appear to play a central role in the initial response to cadmium stress, which involves increasing accumulation.

The addition of Ti-ascorbate to cadmium-containing nutrient solution unambiguously limited the cadmium-induced effect, keeping the sugar content of the plants at the control level, or resulting in a smaller increment than that observed for cadmium alone. The mechanism of titanium in this process is not clear, though data are available on Ti(IV)-induced alterations in catalase, peroxidase, nitrate reductase and phosphofructokinase enzyme activity, and it has also been suggested that titanium plays a role in the photosynthetic electron transport. Our hypothesis is that the first step in the titanium effect could be to modify the Cd^{++} uptake, as suggested by Bedrosian and Hanna (1996).

Without cadmium in the medium the influence of ascorbate differed from that of Ti-ascorbate, causing sugar contents similar to or higher than that induced by cadmium. These data suggest, on the one hand, that in this respect ascorbic acid should be considered as a "stress factor" changing the optimal nutrient composition. On the other hand, the data indicated that, of the components of Ti(IV)-ascorbate, it was Ti(IV) and not ascorbate which played the main role in moderating Cd uptake and thus the toxic effects caused by cadmium.

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CORRELATION BETWEEN NUMBER OF STOMATA AND CONCENTRATION OF MACRO- AND MICROELEMENTS IN SOME WINTER WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES

M. SABO[1], M. BEDE[2] and V. VUKADINOVIĆ[3]

[1]FACULTY OF FOOD TECHNOLOGY, J. J. STROSSMAYER UNIVERSITY, OSIJEK, CROATIA
[2]AGRIGENETICS D. O. O. ENTERPRISE FOR PLANT BREEDING AND SEED PRODUCTION, OSIJEK, CROATIA
[3]FACULTY OF AGRICULTURE, J. J. STROSSMAYER UNIVERSITY, OSIJEK, CROATIA

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The number of stomata and the concentration of macro- and microelements in four new winter wheat genotypes: Lenta, Lara, Perla and Fiesta were investigated in two localities in Croatia in the 1997/98 growing season. The stomata number per mm^2 was determined by a standard method. N was established by the micro-Kjeldahl method, P spectrophotometrically and K, Ca, Mg, Zn, Cu, Fe and Mn by the AAS method. The interrelation of the investigated parameters was determined by multiple regression and correlation analysis. The results obtained indicate that the number of stomata per mm^2 and the macro- and microelement concentrations depended on the genotype, the phenophase and the locality. A statistically significant correlation was found between the stomata number per mm^2 and the macro- (N, P, K, Ca, Mg) and microelement (Zn, Cu, Fe, Mn) concentrations.

Key words: *Triticum aestivum* L., genotype, stomata, macroelements, microelements

Introduction

There are many studies on the number of stomata per mm^2 in plants in general and in wheat in particular (Benkova et al., 1968; Gőbő and Kubjatko, 1971; Teare et al., 1971; Janjatović et al., 1972; Zahirović et al., 1995; Zima et al., 1997) including observations on its dependence on genotype, ecological factors, leaf position and leaf region. Besides these, there is research which points to the effect of macro- and microelement quantities on organ formation, and on the formation of stomata during wheat growth and development (Kastori, 1981) and to the correlation between the concentration of some metals and the chlorophyll content and photosynthetic productivity in new wheat genotypes (Vukadinović et al., 1998). Other investigations indicate the influence of accumulated heavy metals on pigment content, stomatal function and photosynthetic processes (Clijsters and Van Assche, 1985; Van Assche and Clijsters, 1990), the connection between stomatal activity and some metals in the soil (Moustakas et al., 1997) and the positive or negative antagonistic effect of certain metals in wheat leaves (Reilly and Reilly, 1993; Vardaka et al., 1997). However, there are no data available on the correlation between number of stomata and the concentration of macro- and microelements. This fact, as well as the possibility of contributing to a wider knowledge on new genotypes, provided the rationale for the research presented here.

Materials and methods

Four new winter wheat genotypes: Lenta, Lara, Perla and Fiesta from two localities, one close to Donji Miholjac and the other in Kutjevo (Slavonia region), were included in the investigations, which were carried out in the 1997/98 growing season. The wheat genotypes examined differed especially in the grain yield, which averaged 9.7 t/ha in Lenta, 8.4 t/ha in Lara, 8.1 t/ha in Perla and 7.6 t/ha in Fiesta over the two growing sites. The localities chosen differed in the height above sea level, chemical soil properties and climatic conditions. Donji Miholjac is located 86 m.a.s.l. in a distinctly low-lying area, whereas Kutjevo is on undulating ground located 236 m.a.s.l. Donji Miholjac was characterized by soil pH of 7.10 and Kutjevo of 5.70. There were also differences in the P_2O_5 and K_2O contents in the soil, which amounted to 30.56 mg P_2O_5/100 g soil and 14.67 mg K_2O/100 g soil in Donji Miholjac, 15.82 mg P_2O_5/100 g soil and 18.08 mg K_2O/100 g soil in Kutjevo. The mean monthly temperatures varied from –1.1°C (December 1997) to 16.2°C (May 1998) in Donji Miholjac, and from –1.1°C (December 1997) to 15.9°C (May 1998) in Kutjevo. The mean relative air humidity fluctuated from 86% (December 1997) to 66% (May 1998) in Donji Miholjac and from 93% (December 1997) to 69% (May 1998) in Kutjevo. The mean monthly precipitation differed considerably, ranging from 92.4 mm/m^2 (December 1997) to 48.6 mm/m^2 (May 1998) in Miholjac and being significantly higher, ranging from 114.5 mm/m^2 (December 1997) to 91.1 mm/m^2 (May 1998), in Kutjevo.

Sampling for analysis of both number of stomata and macro- and microelement concentrations was done during three phenophases and five stages within the growing season as follows: at tillering in stage 2 (December 1997) and stage 5 (February 1998), at in booting in stage 6 (March 1998) and stage 10 (April 1998) and at heading in stage 10.1 (May 1998). The wheat stages were determined according to Feekes's scale.

Determination of number of stomata per mm^2

Five plants of each genotype were sampled to determine the number of stomata per mm^2. The first leaf (photosynthetically most developed) below the flag leaf was sampled from each plant in 4 replicates, giving a total of 20 leaves. The number of stomata was determined on three leaf regions: top, middle and base, from the ventral side of the leaf. Microscopic preparations were made using the "Collodion" or print method, according to Wolf (1950). The stomata were counted on ten sight fields. The values attained were multiplied with a previously determined area factor (F=0.984) and converted into leaf area units. This parameter was determined with a Carl-Zeiss light binocular microscope at a magnification of 10 (ocular) × 40 (objective).

Determination of macro- and microelements in dry matter of aboveground organs

Twenty plants of each genotype were sampled in 4 replicates, giving a total of 80 plants per genotype. The samples were dried at a temperature of 105°C until reaching constant weight. They were weighed, ground and mixed into an average sample. One gram of sample was digested by Faller's method (Vukadinović et al., 1998). The N concentration was determined from the parent solution by a micro-Kjeldahl method, P spectrophotometrically (660 nm) and K, Ca, Mg, Zn, Fe and Mn by the AAS method (atomic absorbtion spectrophotometry).

Determination of correlation between number of stomata per mm^2 and macro- and microelement concentrations

Differences in the number of stomata per mm^2 and in the concentrations of macro- and microelements between the wheat genotypes at each locality in the various phenophases were investigated by variance analysis and tested with the F-test, using Microsoft Excel 7.0 and Microsoft Word 7.0. The significance of the differences between genotypes, phenophases and localities was determined by the LSD test. ($P_{0.05*;0.01**}$). The interrelation of stomata number and the macro- and microelement concentrations was determined by multiple regression and correlation analysis.

Results

As seen from Figures 1 and 2 the average stomata number in genotype Lenta varied from 39 to 55 per mm^2 at Donji Miholjac and from 43 to 70 per mm^2 at Kutjevo. The smallest average number of stomata was recorded at stage 2 in the first locality and at stage 6 in the second, whereas the largest stomata number was recorded at stage 10.1 in both localities. This genotype (Table 1) was characterized by low concentrations of Ca (0.16; 0.06%), P (0.352; 0.304 %) and Mg (0.64; 0.59%), with a higher value of K (2.64; 2.25%) and the highest for N (2.78; 2.73%) in both localities, though the concentrations of all the elements were lower on average at Kutjevo. The concentration of macroelements, except of Ca, gradually decreased from stage 2 to stage 10.1 at both localities. In the case of the microelements the highest concentrations were recorded for Fe (304.19; 307.77 ppm), followed by Mn (150.10; 125.70 ppm) and Zn (32.61; 29.27 ppm), with the lowest values for Cu (6.83; 8.08 ppm). Again the values were lower in Kutjevo, except for Cu. Unlike the macroelements, the concentration of the microelements tended to increase in later phenophases, except in the case of Zn.

The average stomata number in genotype Lara (Figs. 1 and 2) ranged from 43 to 52 per mm^2 at Donji Miholjac and from 47 to 54 per mm^2 at Kutjevo. The smallest average number of stomata was recorded at stage 5 in Donji Miholjac and at stage 6 in Kutjevo. The largest stomata number at both localities was recorded at stage 10.1. As shown in Table 2 the lowest average macroelement concentrations were found for Ca (0.20; 0.09%), P (0.345%) and Mg (0.73; 0.74%), with higher values for K (2.69; 2.26%) and N (3.09; 2.61%).

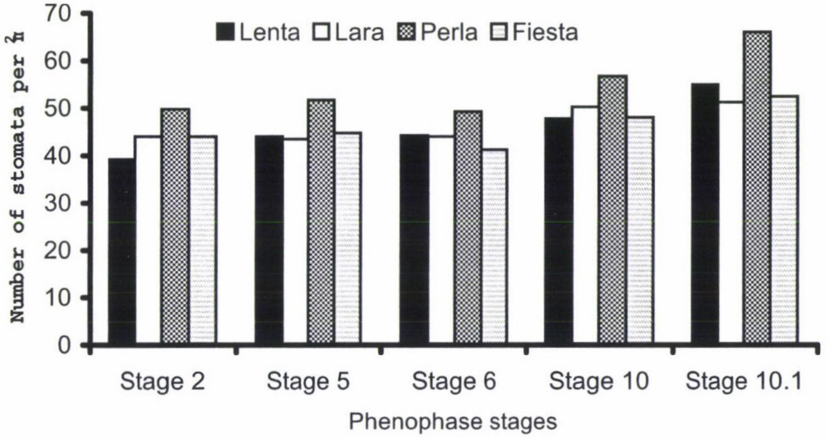

Fig. 1. Average stomata number per mm^2 of leaf area in winter wheat genotypes during the 1997/98 growing season at Donji Miholjac

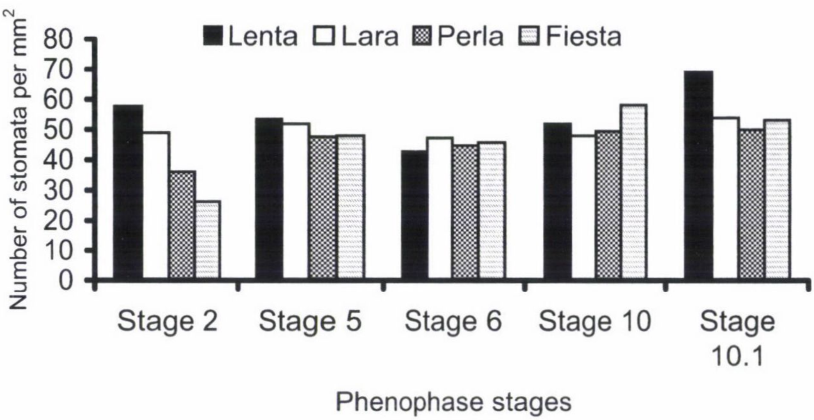

Fig. 2. Average stomata number per mm^2 of leaf area in winter wheat genotypes during the 1997/98 growing season at Kutjevo

Table 1
Average concentration of macro- (%) and microelements (ppm) in the aboveground organs of genotype Lenta in 1997/98 in two localities

Phenophase stages	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
			%				ppm		
					Donji Miholjac				
Stage 2	3.58	0.421	2.92	0.09	0.73	42.75	6.05	264.40	119.00
Stage 5	3.65	0.349	2.37	0.16	0.62	36.95	7.45	136.60	167.50
Stage 6	2.78	0.360	2.70	0.14	0.60	35.35	6.50	539.20	146.00
Stage 10	2.39	0.369	2.89	0.23	0.65	26.25	5.90	126.40	160.00
Stage 10.1	1.52	0.263	2.32	0.18	0.58	21.65	8.25	454.35	158.00
Average	2.78	0.352	2.64	0.16	0.64	32.61	6.83	304.19	150.10
					Kutjevo				
Stage 2	3.64	0.477	2.05	0.07	0.62	42.80	5.49	172.25	81.50
Stage 5	3.62	0.289	2.57	0.05	0.54	39.20	9.30	673.33	160.50
Stage 6	2.58	0.276	2.43	0.11	0.60	26.65	8.65	242.85	171.50
Stage 10	1.84	0.229	2.42	005	0.58	15.70	8.05	234.55	144.50
Stage 10.1	1.95	0.250	1.78	0.04	0.62	22.00	8.90	215.85	70.46
Average	2.73	0.304	2.25	0.06	0.59	29.27	8.08	307.77	125.70

On average the macroelement concentrations of this genotype were lower at Kutjevo than at Donji Miholjac. The concentrations of N, P and K gradually decreased over the phenophases, whereas those of Ca and Mg varied.

Table 2
Average concentration of macro- (%) and microelements (ppm) in the aboveground organs of genotype Lara in 1997/98 in two localities

Phenophase stages	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
			%				ppm		
				Donji Miholjac					
Stage 2	3.97	0.458	2.92	0.13	0.71	57.40	8.10	149.55	140.00
Stage 5	3.55	0.436	2.66	0.20	0.73	41.90	6.80	121.60	133.00
Stage 6	3.38	0.401	2.95	0.28	0.75	40.95	4.60	309.25	128.00
Stage 10	2.55	0.238	2.63	0.19	0.71	31.75	7.20	344.15	132.00
Stage 10.1	2.00	0.278	2.28	0.20	0.77	23.80	8.10	161.90	120.00
Average	3.09	0.362	2.69	0.20	0.73	39.16	6.96	217.29	130.60
				Kutjevo					
Stage 2	3.29	0.440	2.33	0.07	0.65	38.35	3.04	174.00	130.00
Stage 5	3.38	0.403	2.20	0.06	0.56	34.55	4.45	617.90	185.50
Stage 6	3.00	0.309	2.63	0.16	0.72	30.35	8.40	134.20	193.00
Stage 10	1.90	0.351	2.27	0.12	0.62	15.00	7.25	315.55	122.50
Stage 10.1	1.48	0.225	1.88	0.06	0.65	19.10	9.00	245.05	136.00
Average	2.61	0.345	2.26	0.09	0.64	27.47	6.43	297.34	153.40

The highest concentration of the microelements investigated was recorded for Fe (217.29; 297.34 ppm), followed by Mn (130.60; 153.40 ppm), Zn (39.16; 27.47 ppm) and Cu (6.96; 6.43 ppm) with lower values at Kutjevo except for Fe and Cu. The concentration of Zn showed a decreasing trend over the phenophases at both localities, whereas the concentrations of Fe, Cu and Mn varied significantly.

The average number of stomata per mm^2 in genotype Perla (Figs. 1 and 2) varied from 49 to 66 at Donji Miholjac and from 36 to 50 at Kutjevo. The smallest average number of stomata was recorded at stage 6 in the first locality and at stage 2 in the second. The largest number of stomata was determined at stages 10 and 10.1 in both localities. The lowest average concentrations of macroelements in this genotype (Table 3) were recorded for Ca (0.18; 0.08%), followed by P (0.349; 0.288%) and Mg (0.69; 0.63%), with higher values for K (2.73; 2.28%) and N (3.27; 2.59%). The concentrations were lower on average at Kutjevo. Those of N, P and K gradually decreased from stage 2 to stage 10.1, whereas those of Ca and Mg varied. The highest concentration of microelements was found for Fe (246.21; 250.56 ppm), followed by Mn (120.40; 147.10 ppm), Zn (31.25; 24.91 ppm) and Cu (5.83; 7.22 ppm). Unlike Lenta and Lara the concentration of microelements was lower at Donji Miholjac, except for Zn. The concentrations of Zn and Mn tended to decrease, whereas those of Cu and Fe varied considerably.

Table 3
Average concentration of macro- (%) and microelements (ppm) in the aboveground organs of genotype Perla in 1997/98 in two localities

Phenophase stages	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
			%				ppm		
					Donji Miholjac				
Stage 2	3.95	0.428	3.05	0.17	0.74	33.40	5.45	136.85	160.00
Stage 5	3.52	0.329	2.46	0.18	0.59	39.55	3.40	156.95	177.50
Stage 6	3.51	0.380	2.87	0.21	0.70	36.00	6.50	246.15	144.50
Stage 10	3.28	0.305	3.04	0.18	0.74	26.40	6.35	394.50	136.00
Stage 10.1	2.07	0.304	2.21	0.15	0.68	20.90	7.45	296.60	128.00
Average	3.27	0.349	2.73	0.18	0.69	31.25	5.83	246.21	120.40
					Kutjevo				
Stage 2	3.42	0.438	2.31	0.09	0.72	38.50	1.96	157.50	205.50
Stage 5	3.00	0.244	2.19	0.11	0.64	27.65	8.60	145.65	141.00
Stage 6	2.51	0.283	2.55	0.07	0.60	16.00	4.70	214.15	136.00
Stage 10	2.38	0.255	2.63	0.06	0.60	19.15	9.05	439.30	138.50
Stage 10.1	1.63	0.218	1.71	0.05	0.60	23.25	11.80	296.20	115.00
Average	2.59	0.288	2.28	0.08	0.63	24.91	7.22	250.56	147.10

As can be seen from Figures 1 and 2, the average number of stomata in the Fiesta genotype varied from 42 to 52 at Donji Miholjac and from 28 to 58 per mm^2 at Kutjevo. The smallest number of stomata was registered at stage 6 in the first locality and at stage 2 in the second, whereas the largest was found at stages 10.1 and 10 in both. The data in Table 4 reveal that both localities were characterized by low average concentrations of Ca (0.14; 0.07%), P (0.365; 0.277%) and Mg (0.63; 0.59%), with higher values of K (2.71; 2.33%) and N (3.02; 2.63%). As in the previous three genotypes, lower values were recorded in Kutjevo. In both localities the concentrations of N, P and K gradually decreased from stage 2 to stage 10.1, whereas those of Ca and Mg varied from one phenophase to the other. In genotype Fiesta the highest concentration of microelements was recorded for Fe (360.35; 295.94 ppm), followed by Mn (126.60; 152.50 ppm) and Zn (37.60; 26.58 ppm) with the lowest values for Cu (6.99; 7.58 ppm). The concentrations of Cu, Fe and Mn varied considerably, unlike that of Zn which showed a decreasing trend.

The correlation between the stomata number and the macro- and microelement concentrations in the four wheat genotypes investigated was characterized by the values presented in Table 5.

Discussion

Taking into account all the genotypes investigated and both localities, the results presented suggest a decrease in the average stomata number from Lenta to Fiesta, which is thus in positive correlation with the grain yield: the genotype Lenta had the largest number of stomata and the highest yield (9.7 t/ha),

Table 4

Average concentration of macro- (%) and microelements (ppm) in the aboveground organs of genotype Fiesta in 1997/98 in two localities

Phenophase stages	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
			%				ppm		
				Donji Miholjac					
Stage 2	3.69	0.411	2.60	0.09	0.57	48.30	6.35	319.65	140.00
Stage 5	3.79	0.385	3.09	0.24	0.79	41.40	8.20	120.45	143.50
Stage 6	3.54	0.374	2.77	0.18	0.69	35.30	6.25	317.65	147.50
Stage 10	2.65	0.358	2.94	0.10	0.57	29.95	7.60	818.60	158.50
Stage 10.1	1.45	0.298	2.14	0.09	0.54	33.05	6.55	225.40	169.50
Average	3.02	0.365	2.71	0.14	0.63	37.6	6.99	360.35	126.6
				Kutjevo					
Stage 2	3.46	0.454	2.09	0.07	0.64	37.35	3.20	173.75	156.50
Stage 5	3.58	0.274	2.64	0.05	0.62	35.30	9.30	270.05	175.00
Stage 6	2.59	0.255	2.61	0.11	0.64	26.25	8.20	313.75	192.00
Stage 10	1.81	0.227	2.37	0.08	0.52	17.35	7.75	584.05	127.50
Stage 10.1	1.69	0.174	1.93	0.06	0.54	16.65	9.45	138.10	111.50
Average	2.63	0.277	2.33	0.07	0.59	26.58	7.58	295.94	152.50

Table 5

Coefficients of correlation between stomata number per mm^2 and macro- and microelement concentrations in wheat genotypes ($P_{0.05*}$; $P_{0.01**}$) during the 1997/98 growing season

	P	K	Ca	Mg	Zn	Cu	Fe	Mn	S. No.
N	0.852**	0.798**	0.191	0.434**	0.823**	–0.251*	0.149	0.456**	–0.325**
P		0.748**	0.207	0.444**	0.777**	–0.402**	0.104	0.340**	–0.451**
K			0.162	0.470**	0.550**	–0.087	0.310**	0.425**	–0.307**
Ca				0.168	0.335**	0.031	–0.092	0.016	–0.063
Mg					0.401**	–0.195	–0.316**	0.003	–0.233**
Zn						0.268*	–0.014	0.421**	–0.392**
Cu							0.254*	–0.108	0.201
Fe								0.239*	0.211
Mn									–0.283**

S. No.: Stomata number/mm^2

whereas Fiesta had the smallest number of stomata and the lowest yield (7.6 t/ha). The average number of stomata in the genotypes investigated ranged from 26 to 70 per mm^2 of leaf area. A similar positive correlation between stomata number and grain yield was detected in a number of new wheat genotypes in Croatia by Zahirović et al. (1995) and Zima et al. (1997), who found the average number of stomata to be much the same as that determined in the present work. A correlation was also found between the stomata number and the phenophase in all genotypes and in both localities, the smallest stomata number being recorded at stage 2 or 6 and the largest usually at stage 10.1.

The influence of phenophase (F=50.420**) and genotype (F=22.347**) on the stomata number was statistically significant, whereas locality had no significant effect. The locality/genotype interaction, however, was very significant (F=34.732**). The importance of this interaction may be explained by the genetic specificity of the genotype and the influence of ecological factors, in accordance with the conclusion by Janjatović et al. (1972) that wheat stomata number depends on the genotype, stem leaf position and ecological factors. The differences in the average stomata number between the genotypes were also confirmed by the significant interaction between genotype and phenophase stage (F=5.250**) as well as by the locality, phenophase and genotype (F=6.619**) interaction.

As regards the concentrations of macro- and microelements, as a rule, all the investigated genotypes had the highest concentrations of N, P, K, Ca, Mg and Zn at stage 2 in the tillering phase at both localities. However, this trend was not observed for Cu, Fe and Mn.

As the vegetation period proceeded the concentration of the elements decreased due to the intensive accumulation of dry matter, and the direct correlation with the intensity of plant growth was dependent on phenophase, locality and genotype. The genotypes investigated absorbed more macro- and microelements at Donji Miholjac than at Kutjevo (Tables 1, 2, 3 and 4).

The results showed that the correlation between the number of stomata per mm^2 and the concentration of macro- and microelements had predominantly negative values in all genotypes at both localities. A very significant negative influence on stomata number was found for the concentration of N ($P_{0.01^{**}}$), Mg ($P_{0.01^{**}}$) and Zn ($P_{0.01^{**}}$) and a significant effect for Mn ($P_{0.05^{**}}$). By contrast, the correlation between the elements investigated was positive except between Cu and Fe (Table 5).

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TRANSFER OF BLAST RESISTANCE FROM WILD RICE SPECIES INTO CULTIVATED VARIETIES (*O. SATIVA*) WITH ANTHER CULTURE

Q. YANG[1], H. PANG[1], Y. SONG[2] and X. LIU[1]

[1]INSTITUTE OF CROP GERMPLASM RESOURCES, CHINESE ACADEMY OF AGRICULTURAL SCIENCES, BEIJING, CHINA
[2]DEPARTMENT OF AGRONOMY, SHANXI AGRICULTURAL UNIVERSITY, TAIGU, SHANXI PROVINCE, CHINA

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Some wild species of the genus *Oryza* such as *O. rufipogon* and *O. longistaminata* show a high level of resistance to pests and diseases including rice blast (caused by *Magnaporthe grisea*). To transfer blast resistance from wild species into cultivated varieties (*O. sativa*), interspecific hybrids were produced and anther culture was used to accelerate the procedure of resistance breeding. Anther culture efficiency depended on both the medium and the genotype of the cultivated varieties and the wild species. After inoculation with a mixture of six strains with wide spectrum virulence, all the F_1 hybrids were resistant to blast; the F_2 plants segregated, from high resistance to susceptibility, and a similar result was obtained for the H_1 and H_2 plants. At the H_3 stage, blast resistance tended to be stable and almost 100% of inoculated H_5 plants were highly resistant to rice blast. For agronomic characteristics, the F_2 and H_1 showed segregation, but no significant differences were seen between the cultivated parents and the H_2 to H_5 generations. The results demonstrate that blast resistance genes can be transferred from wild rice species into cultivated varieties through crossing and anther culture, and the H_5 can be used as stable lines in future breeding programmes.

Key words: transfer, blast resistance, *Oryza* species, anther culture, *Magnaporthe grisea*

Introduction

Rice blast caused by *Magnaporthe grisea* is a disease of worldwide importance that can greatly decrease rice yield and even totally destroy susceptible varieties under certain environmental conditions. Due to the high cost and environmental considerations of using chemical control, resistance breeding is considered the most promising and practicable approach in the integrated control of this disease. The utilization of disease and pest resistance genes from wild species has become increasingly important in current rice breeding programmes.

The genus *Oryza* consists of approximately 20 wild species and 2 cultivated species. The wild species are spread across tropical and subtropical regions in Asia, Africa, America and Oceania and show a remarkable range of adaptation to different habitats. Their survival in natural habitats over millennia has resulted in adaptation to various biotic and abiotic stresses (Chang, 1985; Vaughan and Sitch, 1991). These wild species are a rich source of useful genes

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for the improvement of cultivated rice, mostly notable for resistance to insect pests and diseases (Mariam et al., 1996). Some germplasm resources of wild species such as *O. rufipogon*, *O. minute* and *O. longistaminata* have been identified as showing strong resistance to *Magnaporthe grisea* (Sun et al., 1992; Jiang et al., 1996). A number of these genes have been transferred from wild species into cultivated rice. For example, genes for resistance to blast from *O. rufipogon*, *O. officinalis* and *O. longistaminata* (Jiang et al., 1996), for resistance to bacterial blight from *O. longistaminata* (Khush et al., 1990) and for resistance to bacterial blight and blast from tetraploid *O. minuta* (Amante-Bordeos et al., 1992) have been transferred to *O. sativa*. These transfers were generally undertaken with traditional breeding methods that take several generations to produce stable lines. Since anther culture produces homozygous genotypes as doubled haploids, it has the advantage of accelerating generation advancement and increasing selection efficiency (Liu et al., 1999). Anther culture for cultivated rice and wild relatives has been well studied. Wu and Kiang (1979) studied anther culture for *O. pernnis* and obtained cultured plants, but at low frequency. Pang (1991) cultured anthers from *O. rufipogon* with an average callus induction ratio of 3.8% and an average shooting differentiation ratio of 4.95%. This research aimed to transfer blast resistance from wild relatives into cultivated varieties of rice via anther culture.

Materials and methods

Plant materials

Varieties of japonica rice: 84-15 and Zhongzuo 9037 (Zh9037) and indica rice: Zhengzhuai (Zh-A) and Changaizhan (Ch-A) were kindly provided by the Institute of Crop Cultivating and Breeding, Chinese Academy of Agricultural Sciences. These varieties were popularly cultivated in China during the 1990s and had characteristics of high yield and good or moderate quality, but they were susceptible to blast. Blast resistance entries of *O. rufipogon* Griff: ST_{221} and S_{7002}, and *O. longistaminata*: CYW_6 were provided by Institute of Crop Germplasm Resources, Guangxi Academy of Agricultural Sciences. In 1993 and 1994, a number of wide crosses were made between these cultivated varieties and wild rice materials and some seeds were successfully harvested for each cross.

Anther culture

Media: a test medium was prepared according to Pang (1987) with the addition of 2 mg/l coenzyme A to N_6 medium. Improved N_6 and MS media with IAA, NAA and KT proved to be the most efficient for callus induction and shooting differentiation, respectively, among the six tested media.

Callus induction: young panicles of F_1 were selected, wrapped in wet gauze and stored at 10±2°C for preconditioning. Three to twenty days later, the preconditioned young panicles were disinfected with 5% NaClO for 5 minutes and rinsed with nanopure water 6–7 times, after which the anthers were placed in improved N_6 medium at 25–30°C in a dark room.

Shooting differentiation: calli from the induction culture were cut into 2–2.5 mm long segments, translocated onto shooting differentiation media (MS with 0.2 mg/l IAA, 0.5 mg/l NAA and 0.8 mg/l KT) and cultured at room temperature (25–28°C) under 2,000–3,000 Lux light for 14 h a day.

Resistance evaluation

Pathogen cultures were prepared using the same method as Ling (1984). The *Magnaporthe grisea* strains 95-t_2, 90-29a, ZJ91-11, 91-17-2 and 91-78-1 were used to test for blast resistance in the parents, F_1, F_2 and H_1–H_5 plants. The H_1 originated from doubled haploid plants and the H_2 plants were self-pollinated H_1 identified as having high resistance to rice blast. The H_3–H_5 plants were chosen similarly. Fresh cultures of the above strains were revived from colonized dry paper disks maintained at 4°C. For the evaluation of blast resistance, plants at the 3–4 leaf stage from the parents, F_1, F_2 and H_1–H_5 progenies were inoculated by spraying a mixed spore suspension (30–50 spores per 100x micro-vision) of strains 95-t_2, 90-29a, ZJ91-11, 91-17-2 and 91-78-1. After inoculation with the strains, the plants were incubated in dew chambers at 24°C for 24 h and then moved to a glasshouse. After 10–12 days, disease reactions were scored according to lesion types (Ling, 1984).

Lesion types:

1. b: brown speck
2. bg: less than 2 mm long lesions with brown margins
3. bG: more than 2 mm long lesions with brown margins
4. pG: more than 2 mm long lesions with pale or purple margins

Scores:

1. no evidence of infection	R^h
2. b>bg+bG+pG and bG+pG=0	R
3. b>bg+bG+pG and bG+pG≥1	MR
4. b≤bg+bG+pG and b+bg>bG+pG	MR
5. b≤bg+bG+pG and b+bg<bG+pG, bG>pG	MS
6. b≤bg+bG+pG and b+bg<bG+pG, bG<pG	S

Results

Analysis of anther culture efficiency

Anther culture efficiency is determined as callus induction and shooting differentiation efficiency. Callus induction ratios varied between 1.696% and 3.848% and the shooting differentiation ratios were from 15.8% to 40.32% for different wide crosses (Table 1). In the same culture conditions, different genotypes of cultivated rice and wild species showed significant differences in both measures. The callus induction ratio (3.85%) of S_{7002}/Ch-A was much higher than the others, while the combination of 84-15/CYW_6 showed the highest shooting differentiation ratio (40.32%). The combinations 84-15/ST_{221} and 84-15/CYW_6 had the same maternal parent (cultivated varieties) but different paternal parents (wild species), and their callus induction efficiencies and shooting differentiation efficiency were very different. On the other hand, the same paternal combinations of 84-15/ST_{221} and Zh-A/ST_{221} also showed differences in callus induction and shooting differentiation efficiency.

Evaluation for blast resistance

The blast resistance of the parents had been previously identified. All entries of wild rice were highly resistant and the varieties of cultivated rice varieties were susceptible to blast. Their F_1 hybrids, resistant to blast, showed uniformity. The F_2 segregated clearly from highly resistant to susceptible. For anther cultured progeny, the ratio of resistant plants to susceptible plants increased quickly from H_1 to H_5. Less than 50% of H_1 and H_2 inoculated plants were resistant to blast and only 9.26–13.04% were highly resistant. However, from H_3 all inoculated plants were resistant to blast though some showed only moderate resistance. Highly resistant plants made up more than 90% in H_3. Almost all tested materials at H_4 and H_5 showed high resistance, and little change was observed between these two generations (Table 2). The blast disease reactions for all combinations had the same trend and no great difference occurred among the genotypes of cultivated varieties and wild species.

Table 1

Comparison of anther culture efficiency of wide crosses between different cultivated and wild rice genotypes

Cross	No. of cultivated anthers	Callus induction		Transferred calli	Shooting differentiation	
		No. of calli	%		No. of shoots	%
84-15×ST_{221}	2890	49	1.696	40	11	27.50
84-15×CYW_6	2880	81	2.810	62	25	40.32
Zh-A×ST_{221}	3180	64	2.010	44	14	31.82
S_{7002}×Ch-A	3768	145	3.848	120	19	15.80

Table 2

Percentage of blast-resistant progenies of anther-cultured crosses from wild and cultivated rice resources

Cross	Generation	No. of inoculated plants	R^h		R		MR	
			Plants	(%)	Plants	(%)	Plants	(%)
84-15 × ST_{221}	H_1	34	2	5.88	4	11.76	3	12.50
	H_2	54	5	9.26	4	7.41	8	14.81
	H_3	25	23	92.00	1	4.00	1	4.00
	H_4	25	24	96.00	1	4.00		
	H_5	28	28	100				
84-15 × CYW_6	H_1	32	3	9.38	2	6.25	1	3.13
	H_2	46	6	13.04	3	6.52	5	10.87
	H_3	30	28	93.33	2	6.67		
	H_4	30	29	96.67			1	3.33
	H_5	25	25	100				
Zh-A×ST_{221}	H_1	28	3	10.71	6	21.43	3	10.71
	H_2	58	5	8.62	8	13.79	5	8.62
	H_3	25	23	92.00	1	4.00	1	4.00
	H_4	30	29	96.67	1	3.33		
	H_5	25	25	100				
S_{7002}×Ch-A	H_1	36	2	5.56	1	2.78	5	13.89
	H_2	47	5	10.64	6	12.77	3	6.38
	H_3	42	39	92.86	2	4.76	1	2.38
	H_4	30	28	93.33	2	6.67		
	H_5	30	29	96.67	1	3.33		

Stability of agronomic characteristics among anther-cultured progeny

H_1 anther-cultured progeny separated significantly and showed abundant diversity for almost all characteristics measured, including plant height, plant architecture, stigma colour, spike type, awns, length and width of flagleaf, length of anther, seed shape, glume colour, seed coat colour, shattering and growth period (Table 3). Some characters could be related to the maternal (cultivated varieties) or paternal parents (wild species), others had characteristics of both parents and a few were transgressive. For instance, the growth period (115–168 d), plant height (30–145 cm), number of grains per spike (35–215) and weight per 1000 grains (18–28 g) of 147 plants analysed from 45 plots of Zh9037/ST_{221} varied over a wide range. During the growth period, some plants were dwarfed with small narrow leaves, and although they flowered they had no seed set or died early. These plants may have been haploid.

The characteristics of the H_2 generation appeared to be stable. Table 3 shows some statistical data from the H_2, H_3, H_4, H_5 and F_2 of the 84-15/ST_{221} cross. No significant difference was seen for plant height, flagleaf length, flagleaf width, spike length, grains per spike or growth period among the H_2, H_3, H_4 and H_5. Interestingly, the maximum, minimum and average of grain number per spike of the cultivated variety (84-15) were 167, 200 and 189, while those of the wild rice (ST_{221}) were 18, 57 and 34, respectively. However, these characteristics were greater than both parents for the H_2, H_3, H_4 and H_5 generations. The transgression might result from the heterosis of the distant cross.

Discussion

Useful genes from wild species can be transferred into cultivated varieties using different approaches. Jena and Khush (1990) successfully transferred resistance to brown planthopper and whitebacked planthopper from *O. officinalis*; Amante-Bordeos et al. (1992) transferred bacterial blight and blast resistance from *O. minuta*. The results in this study demonstrate that anther culture can be used to accelerate stabling the transferred blast resistance genes from *O. rufipogon* and *O. longistaminata*. For all crosses attempted, highly resistant lines were derived, even though the cultivated parents were all blast-susceptible.

It is well known that wild rice species are rich sources of tolerance or resistance to a number of biotic or abiotic stresses such as diseases and pest insects, drought and cold. Distant crosses have been successfully performed between species of *Oryza*, but because of the genetic barriers, most of the successful cases reported were observed between species in the same chromosome set. To transfer useful genes to cultivated varieties especially from different chromosome set wild species, techniques such as embryo rescue, DNA transfer through pollen tubes and even gene transformation should be used to improve the transfer effectiveness.

Table 3
Analysis of some characters of pollen plants, their parents and F_2 hybrids from 84-15/ST_{221}*

		84-15	ST_{221}	F_2	H_2	H_3	H_4	H_5
Plant height (cm)	Min	87.40	102.00	76.00	84.20	88.50	88.50	81.50
	Max	100.00	136.00	127.60	112.00	106.00	106.00	103.00
	$\overline{X}$	96.41	120.43	97.51	96.03	97.06	96.75	96.12
	S	3.70	11.90	12.13	6.70	5.06	4.64	5.04
	C.V.%	3.848	9.88	12.44	6.98	5.21	4.80	5.24
Flagleaf length (cm)	Min	24.20	25.20	21.00	27.80	27.80	28.00	27.00
	Max	37.80	47.50	37.50	35.80	34.10	34.00	33.00
	$\overline{X}$	30.99	39.63	29.60	30.29	30.14	30.37	30.10
	S	2.57	6.21	4.81	1.78	1.74	1.70	1.65
	C.V.%	8.29	15.67	16.25	5.87	5.78	5.59	5.48
Flagleaf width (cm)	Min	1.10	0.30	0.40	0.70	0.70	0.70	0.80
	Max	1.70	0.60	1.50	1.50	1.50	1.50	1.50
	$\overline{X}$	1.45	0.57	0.94	1.07	1.10	1.08	1.07
	S	0.18	0.15	0.35	0.22	0.23	0.21	0.18
	C.V.%	12.24	26.22	37.65	20.85	21.08	19.81	17.12
Spike length (cm)	Min	18.70	10.50	14.60	17.00	18.40	17.90	15.00
	Max	25.80	17.60	24.00	23.00	23.00	20.00	23.00
	X	20.50	14.09	19.02	20.20	20.19	20.07	19.53
	S	1.65	2.72	2.73	1.25	1.13	1.33	1.77
	C.V.%	8.04	19.30	14.34	6.20	5.62	6.60	9.08
No. of grains/ spike	Min	167.00	18.00	124.00	182.00	173.00	173.00	167.00
	Max	200.00	57.00	223.00	231.00	214.00	214.00	217.00
	$\overline{X}$	189.43	34.20	173.27	198.27	197.00	195.80	195.37
	S	8.17	13.32	33.14	11.40	10.58	12.06	13.99
	C.V.%	4.31	38.96	19.33	5.75	5.37	6.16	7.16
Growth period (d)		145	perennial	perennial$^+$	143	142	143	142

*All data in this table were from 30 investigated plants; $^+$or 140–145

Wild rice species possess many undesirable agronomic and quality characteristics, and extensive backcrossing is usually required to retain superior and discard inferior characters (Jiang et al., 1996). If the wild rice parent shows a high level of resistance and the heritability of this trait is high, hybrids formed with cultivated varieties showing good agronomic characteristics and high yield can be rapidly bred through anther culture of the F_1 hybrid.

Reliability of resistance selection with strain mixtures covering a wide spectrum of virulence

The emergence of strains with wide spectrum virulence in northern China meant that almost all cultivated rice varieties were susceptible to blast. Inoculation with mixtures of strains has proved successful for selecting blast resistance germplasm. This was tested by Jiang et al. (1996) who used 7 Japanese representative strains and a mixture of 6 strains showing a wide spectrum of virulence to inoculate 334 entries of wild rice. The results of the two procedures were similar and 25 blast-resistant accessions of wild rice were identified.

One would have expected that resistance selection from H_1 and H_2 would show similar stable resistance due to their genetic homologies. However, H_2 plants still showed a resistance spread from highly resistant to susceptible. This result might be caused by the following: 1) physiological variations occurred during the period from callus to shooting and during the period of doubling, but these variations proved uninheritable (Sun, 1978); 2) since the inoculation was done with a mixture of 5 strains, the dominant strains are different in H_1 and H_2; some strains might be more virulent in H_1 and others in H_2. Significantly, from H_3, all plants were resistant and the resistance was stable. Therefore, anther-cultured progeny of cultivated varieties × wild species could be selected from H_1 by harvesting the seeds from different plants separately, but selection in H_2 is more important for obtaining pure lines.

Comparison between anther culture and traditional breeding methods

The cross-pollination of some popularly used wild rice species such as *O. sativa* f. *spontanea* results in their genetic heterozygosity. The progeny of wild rice species and cultivated varieties segregate widely and it takes a long period (generally 8–10 generations) to make them homozygous. Moreover, the strong heritability of some inferior characteristics of wild rice species makes it more difficult to obtain stable lines with the target genes and both good quality and high yield. Anther culture has the advantage of making the hybrid progeny homozygous and of discarding undesirable characteristics quickly. It takes only 3–5 generations to obtain the expected stable lines. Considering the cheap equipment and chemicals required for anther culture compared with the field and labour needs of traditional breeding, anther culture is a time- and cost-saving method of rice breeding.

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GENETIC RELATIONSHIPS BETWEEN GRAIN YIELD AND YIELD COMPONENTS IN A SYNTHETIC MAIZE POPULATION AND THEIR IMPLICATIONS IN SELECTION

N. VASIC[1], M. IVANOVIC[1], L. A. PETERNELLI[2], D. JOCKOVIC[1], M. STOJAKOVIC[1] and J. BOCANSKI[1]

[1]RESEARCH INSTITUTE OF FIELD AND VEGETABLE CROPS, NOVI SAD, YUGOSLAVIA
[2]UNIVERSIDADE FEDERAL DE VICOSA, VICOSA, BRAZIL

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The synthetic maize population 316PO2 was subjected to genetic correlation analyses between grain yield, yield components and morphological traits. The purpose was to enable estimates to be made of the advantage of using selection indices compared with selection based on grain yield only, and if that advantage was present, to choose enough simple selection indices for practical use. Selection indices were constructed out of four traits highly significantly correlated with grain yield, in addition to yield itself.

Grain yield exhibited a highly significant additive genetic correlation with ear diameter (r_a=0.588**), kernels row^{-1} (r_a=0.643**), ears plant^{-1} (r_a=0.871**) and ear height (r_a=0.427**). The most efficient index was Index No. 14 (R.E.I_{12345}= 108.83%), which included all four traits and grain yield. Index No. 3, one of the simplest forms of index, including only ears plant^{-1} and grain yield, showed slightly less relative efficiency (R.E.I_{35}=107.24%) than Index No. 14. Using this simple form of index with two characters (Index No. 3) could improve the efficiency of selection for grain yield. The estimated advantage from its use is 179.6 kg/selection cycle for grain yield over selection based only on grain yield.

Key words: maize, synthetic population, genetic correlation, selection index

Introduction

Grain yield is the most economically important trait in maize, and its heritability is the lowest of all traits, generally less than 30% (Hallauer and Miranda, 1988). Using some other trait that is highly correlated with grain yield and has higher heritability as an auxiliary trait along with grain yield in recurrent selection programmes should make the selection of the best progenies, those with the highest yield potential, more reliable.

What is meant by the term auxiliary trait? Indirect selection for a trait as complex as yield is not plausible. One of the reasons is that the genetic correlation of traits with grain yield is frequently too small to compensate for greater heritabilities. Another important reason is that grain yield is an expression of fitness and drastic changes in one component of yield are accompanied by adjustments in other components (Hallauer and Miranda, 1988). For that reason, the aim was to include one or more traits besides grain yield in the process of selection for grain yield in order to increase selection efficiency.

Correlation coefficients (Fisher, 1918) are of interest but of little value unless some application is made of the various relationships. Also, the inclusion of various characters in selection programmes is often not practicable. The best way to achieve a simultaneous improvement in grain yield and one or more auxiliary traits is to use selection indices. At the same time, the indices provide an objective approach to the use of data from correlation analyses. The phenotypic and genotypic variances and covariances used to compute the correlation coefficients provide the basis for constructing a selection index that gives proper weight to each character included in the index (Robinson et al., 1951).

The mating design used in the present study allowed total genetic variation to be separated into additive and dominance components. Additive genetic variances and covariances, as the heritable portion of total genetic variation, were used to compute correlation coefficients and selection indices.

The objective of this study was to determine a simple selection index including grain yield and one or more auxiliary traits in order to increase grain yield selection efficiency in a yellow dent maize synthetic population.

Materials and methods

The yellow dent maize synthetic 316PO2 was used as the base population for the study. The population had a maturity rating of FAO 600. The 316PO2 population was developed from 15 inbred lines. A complete diallel cross was used to intermate these 15 lines, and three generations of random mating were completed before beginning the study. The random mating was done in an isolation field.

Using the Design I mating system (Nested Design), introduced by Comstock and Robinson (1948), randomly selected male plants were crossed to randomly selected female plants (each male plant was crossed to four female plants). This resulted in 280 full-sib families belonging to 70 half-sib groups. Delayed planting of a portion of the population from which the male plants were chosen was used to avoid assortative mating (Lindsey et al., 1962).

Progenies were tested during 1996 and 1997 in field experiments at two locations per year in an incomplete block design (Cochran and Cox, 1992). The test locations were Rimski Sancevi (41°N) and Srbobran (42°N), both in a semiarid climate. The soil in Rimski Sancevi is a partially hydromorphic type of carbonate chernozem. In Srbobran the soil is of the alluvial deposit type. These two soil types are dominant in the main maize growing area in Yugoslavia. The standard maize growing techniques were applied, without irrigation.

The 70 male groups were assigned at random to 14 sets. Each set, therefore, contained five half-sib families or 20 full-sib families. Three replications within each set were used and 14 plants per plot were grown at a density of 57,000 plants ha^{-1}. The plots were overplanted with double density and thinned at the 5-leaf stage. Each plot consisted of one row, spaced 70 cm apart. The data were recorded on 10 competitive plants (plants in the middle of the plot, excluding end of plot plants) for grain yield, ear length, ear diameter, kernel depth, 1000-kernel weight, ear row number, kernels row^{-1}, ears $plant^{-1}$, plant and ear height. Harvesting was done by hand.

The combined analysis of variance pooled over sets and repeated over environments for plot averages and individual characters was performed as suggested by Cockerham (1963) and Hallauer and Miranda (1988).

Total and additive genetic correlations between traits were calculated as the covariance between traits divided by the square root of the product of the corresponding individual trait variances. The significance of the correlation coefficients was tested by the *t* test using the standard error of the corresponding coefficient. The standard errors of the correlation coefficients were computed as suggested by Falconer (1996) using the correction factor

$$SE_r = \frac{1-r}{\sqrt{2}} \sqrt{\frac{SE_{h_x^2} SE_{h_y^2}}{h_x^2 h_y^2}}$$

The basis for the development of selection indices was presented by Hazel (1943) with an example in animals and by Smith (1936) who illustrated its use in plants. Various selection indices for grain yield, the genetic advance expected from their use and their relative efficiency were computed in the manner described by Robinson et al. (1951). The expected genetic advance was calculated for a selection intensity of 5%. When constructing the selection indices all combinations of grain yield and traits which expressed highly significant additive correlations with grain yield were included. The expected genetic progress per cycle, when selection was based on yield alone, was calculated according to the formula given by Hallauer (1980): $\Delta_G = k\, \sigma^2_A / \sigma_P$, where k is the standardized selection differential in a standard deviation unit, σ^2_A is the additive genetic variance and σ_P is the phenotypic standard deviation.

Results

Grain yield exhibited highly significant (P>0.01) positive additive genetic correlations with ear diameter (r_a=0.588), kernels row^{-1} (r_a=0.643), ears plant^{-1} (r_a=0.871) and ear height (r_a=0.427) (Table 1). Total genetic correlation coefficients between grain yield and other examined traits were highly significant, except for 1000-kernel weight, which showed the lowest genetic association with grain yield at both levels. Some of the traits, such as ears plant^{-1} and ear height, exhibited very uniform additive and total genetic correlations with grain yield. The total genotypic correlations were higher than the corresponding additive correlations in most cases (Table 1).

Since only ear diameter, kernels row^{-1}, ears plant^{-1} and ear height exhibited very significant additive correlations with grain yield, these four traits proved to be the most important components of grain yield. The genetic gain per cycle and the relative efficiency of 16 selection indices constructed out of these four characters and grain yield are given in Table 2.

In Table 2 the top line gives the expected genetic progress when selection is based on yield alone and this is then used as a basis for the comparison of the relative efficiency of using the various selection indices. Index No. 14, which involved all five characters, gave the highest relative efficiency of selection (R.E.I_{12345}=108.83%). This was followed by Index No. 11 and index No. 6, which involved three characters – ear diameter, ears plant^{-1} and yield (R.E.I_{135}=108.36%). Index No. 3, which was one of the simplest forms of index involving only two characters, showed 7.24% higher efficiency than straight selection for grain yield, or 179.6 kg/cycle more genetic gain (Table 2). On the other hand, Index No. 3 was only 1.59% less efficient than the top-ranking Index No. 14, with 39.6 kg/cycle less genetic gain.

Table 1

Additive genetic correlations (above diagonal) and total genetic correlations (below diagonal) among traits in maize synthetic population 316PO2

Traits	1	2	3	4	5	6	7	8	9	10
1	–	0.350	0.588**	0.332	0.227	0.256	0.643**	0.871**	0.273	0.427**
2	0.355**	–	-0.028	-0.051	0.159	-0.147	0.531**	0.313	0.342	0.435*
3	0.388**	-0.208*	–	0.536**	0.425*	0.407*	0.261	0.133	0.409*	0.287
4	0.409**	-0.404**	0.612**	–	0.432**	0.026	0.216	0.001	0.245	0.351*
5	0.017	0.242**	0.202**	0.086	–	-0.410*	-0.356*	0.088	0.366*	0.340
6	0.211**	-0.224**	0.509**	0.254*	-0.490**	–	0.174	-0.046	0.062	-0.094
7	0.557**	0.566**	-0.015	0.035	-0.344**	-0.009	–	0.581**	0.132	0.308
8	0.894**	0.193*	0.129	0.259*	-0.109**	0.075	0.398**	–	0.029	0.299
9	0.365**	0.318**	0.333**	0.329**	0.273**	0.042	0.189*	0.236**	–	0.694**
10	0.425**	0.338**	0.173*	0.175	0.129**	-0.026	0.284**	0.349**	0.732**	–

1: Grain yield, 2: Ear length, 3: Ear diameter, 4: Kernel depth, 5: 1000-kernel weight, 6: Ear row number, 7: Kernels row^{-1}, 8: Ears plant^{-1}, 9: Plant height, 10: Ear height; *, ** Significant at the 0.05 and 0.01 probability level, respectively.

Table 2

Expected genetic gain per cycle in grain yield ($\Delta G/c$) from the use of selection indices and their relative efficiency (R.E.) in synthetic maize population 316PO2

Index number	Index	$\Delta G/c$ (t/ha)	R. E. (%)
	Grain yield	2.4820	100.00
1	I_{15}	2.5911	104.39
2	I_{25}	2.4820	100.00
3	I_{35}	2.6616	107.24
4	I_{45}	2.4646	100.11
5	I_{125}	2.6183	105.49
6	I_{135}	2.6895	108.36
7	I_{145}	2.5920	104.43
8	I_{235}	2.6640	107.33
9	I_{245}	2.4931	100.45
10	I_{345}	2.6670	107.53
11	I_{1235}	2.6989	108.74
12	I_{1245}	2.6183	105.49
13	I_{2345}	2.6700	107.58
14	I_{12345}	2.7012	108.83
15	I_{13}	0.8877	35.76
16	I_{123}	0.9544	38.45

Traits in index: (1) ear diameter, (2) kernels row^{-1}, (3) ears plant^{-1}, (4) ear height, (5) grain yield

Indices Nos. 2, 4 and 9, which included kernels row^{-1}, height of ear and grain yield, had about the same level of genetic gain as straight selection for grain yield. This result confirms that in spite of highly significant additive correlations of kernels row^{-1} and height of ear with grain yield, these two traits did not actually make much contribution to the formation of grain yield. The results of path coefficient analysis (Wright, 1921, 1923) (data not shown) also confirmed this statement. The direct effect of kernels row^{-1} and height of ear on the grain yield was very small and not significant.

Indices from which grain yield was excluded as a constitutive trait led to considerably less genetic gain and hence less relative efficiency. Only two indices of this type are demonstrated: No. 15 and No. 16 (Table 2).

Discussion

Grain yield exhibited the highest total genetic and additive genetic correlations with ears plant^{-1} (r_g=0.894**, r_a=0.871**), which is in accordance with the results of Robinson et al. (1951), Stuber et al. (1966), Lindsey et al. (1962) and Arias et al. (1999). The estimated additive genetic correlation between ear diameter and grain yield (r_a=0.588**) was similar to that reported by Bartual and Hallauer (1976). The additive genetic correlation between these two traits was higher than the total genetic correlation. This was a result of the small difference between the additive and total genetic covariance of these traits in favour of total genetic covariance, as well as of the much higher total genetic variance of grain yield compared to the additive genetic variance for the same trait (data not shown). Similar results were reported by Silva (1974). The same situation was observed for the correlation between kernels row^{-1} and grain yield.

Index No. 14 expressed the highest advantage in expected genetic gain over straight selection for grain yield (219.2 kg/cycle). In practical breeding the use of an index constructed from five different traits would be complicated and time- and money-consuming, reducing the gain attainable by its use. If a selection index is to be recommended for use in practice it should be both efficient and easy to use (Smith et al., 1981). The use of a simple index such as Index No. 3, which does not require any additional costs of selection, would raise the genetic gain for grain yield by 7.24%, or 179.6 kg/selection cycle. The efficiency of selection indices that involve ears plant^{-1} in addition to grain yield was also confirmed by the results of Robinson et al. (1951) on various F_2 populations of two inbred lines as well as by the findings of Parh et al. (1988) in exotic open-pollinated maize varieties.

Ears plant^{-1} is a trait that is easily recorded during hand harvesting (as was the case in this experiment) and requires no additional effort. Index selection using the constructed index No. 3 should increase both grain yield and the number of ears plant^{-1}, or prolificacy. Increased prolificacy reduces the risk of stress and the genotype × enviroment interactions, i.e. the flexibility of the maize plant is increased for making adjustments to a stress situation (Hallauer and Troyer, 1972). Stress situations are frequent in semiarid growing conditions, mainly caused by drought at flowering time, which is common in this region. The consequence of stress at this period of plant life is very often the appearance of barren plants.

Index No. 6 was 1.12% more efficient than Index No. 3, or 27.9 kg/selection cycle, but many more observations and measurements were required than for Index No. 3. For this reason, Index No. 3 is now being used in a recurrent selection programme in the studied population 316PO2 for the simultaneous improvement of grain yield and prolificacy.

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TOLERANCE OF SORGHUM LANDRACES AND VARIETIES TO STRIGA (*STRIGA HERMONTHICA*) INFESTATION IN ETHIOPIA

W. BAYU, S. BINOR and L. ADMASSU

SIRINKA AGRICULTURAL RESEARCH CENTER, P. O. BOX 74, WELDIA, ETHIOPIA

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A pot experiment was conducted at Sirinka Agricultural Research Center, Ethiopia in 1999 to evaluate the level of resistance of local and improved sorghum varieties to *Striga hermonthica* (Del.) Benth. The results indicate that the three exotic varieties, which were bred for striga resistance (P-9401, P-9403 and SRN-39), and two local varieties (Ayefere-Asfachew and Wotere) supported significantly lower numbers of emerged striga compared to the susceptible checks. Striga dry biomass weight and shoot height were also significantly lower for these varieties. Plant height, dry shoot weight and dry root weight were also least affected by striga infestation in these varieties. Most of the local sorghum varieties, which were praised for their resistance, had disappointing infestation levels.

Key words: striga, resistance, genotypes, landraces, exotic varieties

Introduction

Sorghum (*Sorghum bicolor* L.) Moench] is the major food crop in north-eastern Ethiopia. However, the production of this important crop is threatened by the parasitic weed striga [*Striga hermonthica* (Del.) Benth.]. The weed is a root parasitic plant incurring huge yield losses in sorghum, maize and millet in vast areas of north-eastern Ethiopia. The attack is so severe that farmers cannot grow sorghum any more. In some localities farmers have either abandoned their land or switched to other minor crops. For instance, Esilaba et al. (1998) indicated that about 3.9% of the farmers in Welo had abandoned land due to heavy striga infestation. Hence, striga represents the largest biological barrier to sorghum production in the entire sorghum-growing areas of north-eastern Ethiopia. The control of striga has not been easy at all. Several control measures have been tried but economically effective means of control are not yet available (Parker and Riches, 1993). Breeding resistant genotypes is the most promising, practical and economical approach to reduce the damage caused by striga (Ejeta et al., 1993; Hess et al., 1991; Oliver et al., 1991). The existence of sorghum varieties presenting some degree of resistance has often been reported (Doggett, 1988; Obilana et al., 1991; Oliver et al., 1991; Ramaiah, 1984). Ejeta et al. (1993) have also reported that sorghum genotypes differ as much as a billionfold in the amount of striga germination stimulant they produce. Several sorghum varieties that showed relatively consistent resistance were identified in Ethiopia (Ramaiah, 1984; Anonymous, 1994). However, it was also reported (Mulatu and Kebede, 1991) that most of these varieties had poor agronomic qualities.

Most of the efforts made in this line in Ethiopia were confined to exotic genotypes. No or little attempt has been made to use the local genetic pool,

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despite the fact that Ethiopia is the centre of diversity and that farmers often claim the existence of striga-resistant sorghum landraces. A pot experiment was therefore conducted with the objective of evaluating the levels of resistance in local sorghum varieties which were claimed to be resistant to striga. The knowledge gained from this finding would suggest which sorghum varieties could be utilised or rejected for cultivation under striga infestation, in addition to the opportunity of transferring resistance to high-yielding varieties.

Materials and methods

A pot experiment was conducted at Sirinka Agricultural Research Center in 1999 with 15 sorghum lines. The experiment was laid out in a randomized complete block design with three replicates. Triplicate pots of soil infested with striga seeds were used for each sorghum line, while another set of triplicate striga-free pots were used as control. This experiment was conducted in the open where the pots were arranged with 30 cm spacing. Table 1 gives a description of the sorghum varieties included in this study, which tried to include only those varieties that were praised by farmers for their striga resistance. The local variety Degalit and the exotic line Key # 8574 were used as susceptible checks. Degalit is the most popular, productive variety (in striga-free plots) in north-eastern Ethiopia. All the long-cycle and medium-cycle lines were local varieties, whereas all the short-cycle varieties were exotic varieties, among which P-9401 and P-9403 were initially included as test entries but were released as striga-resistant varieties after the initiation of this experiment. Therefore, these two varieties could be considered as standard checks in this study.

Free-draining plastic pots of 30 cm top diameter, 20 cm bottom diameter and 30 cm height were used. The pots were filled with a 1:1 mixture of soil and sand and about 150 mg of *S. hermonthica* seeds were mixed in the top 5 cm of the soil before sowing. Five sorghum seeds were sown on April 6 and thinned to two and one plant one and two weeks after emergence, and watered as needed to avoid moisture stress. No fertilizer was applied to the pots. The experiment was terminated after 150 days. The number of emerged striga was used as the main index of resistance. Data on the shoot and root biomass and plant height development of each entry were collected. Dry sorghum shoot and root weight and striga biomass weight were recorded by drying in the oven at 75°C until constant weight was obtained. Sorghum and striga shoot height was measured at harvest. Emerged striga shoots were counted thrice during the course of the experiment. Analysis of variance for the measured parameters was performed using the MSTATC statistical computer program (MSTATC, 1989).

Table 1
Description of sorghum varieties included in the study

Identification	Local name	Maturity group
SSGM 022	Kindibe Tikur Cherekit	Medium-cycle
SSGM 033	Mog Ayefere	Long-cycle
SSGM 037	Minchiro	Long-cycle
SSGM 050	Kindibe Nech Cherekit	Medium-cycle
SSGM 063	Merhabete	Medium-cycle
Kitign Ayefere	Kitign Ayefere	Long-cycle
Ayefere-Asfachew	Ayefere-Asfachew	Medium-cycle
P-9401	–	Short-cycle
P-9403	–	Short-cycle
SRN-39	–	Short-cycle
Wotere	Wotere	Medium-cycle
Merare	Merare	Long-cycle
Jigurti	Jigurti	Medium-cycle
Degalit	Degalit	Long-cycle
Key # 8574	–	Short-cycle

Results and discussion

Striga count, striga biomass weight and striga shoot height

There were significant (P<0.01) varietal differences in the number of striga shoots supported, striga dry biomass weight and striga shoot height (Table 2). According to Ejeta et al. (1993) a genotype is said to be resistant when it supports significantly fewer striga plants and has a higher yield than a susceptible cultivar when it is grown under striga infestation. Similarly, Obilana (1984) defined resistance as a "low total number of striga per sorghum plant". In the present study the sorghum varieties P-9401 and SRN-39 supported a significantly lower number of emerged striga shoots (0.12 and 0.35) as compared to the susceptible checks (1.44 and 1.62). Similarly, the two local varieties Ayefere-Asfachew and Wotere supported a significantly lower number of striga shoots compared to the susceptible checks. The mean numbers of striga supported by these varieties were comparable to that supported by one of the varieties bred for striga resistance (P-9403). These varieties supported 0.71 and 0.77 striga plants as compared to 1.44 and 1.62 striga plants in the susceptible checks and 0.12 and 0.68 in the resistant checks (P-9401 and P-9403). The growth and vigour of the plants as indicated by aboveground shoot weight and plant height was least affected by striga in Ayefere-Asfachew and Wetere. The highest number of emerged striga plants was supported by the susceptible check Key # 8574 followed by Degalit, Merare, Jigurti and SSGM-037. The rest of the sorghum varieties did not differ from the susceptible checks (Degalit and Key # 8574) with regard to striga shoot count.

Dry striga biomass weight followed the trend in striga count, with significantly (P<0.01) higher striga biomass weight recorded in the susceptible checks. Significantly lower striga biomass weight was recorded in the exotic varieties (P-9401, P-9403 and SRN-39) and the two local varieties (Ayefere-Asfachew and Wotere). The dry striga biomass weight in Ayefere-Asfachew and Wotere was 0.36 g compared to 1.33 and 1.37 g in the susceptible checks and 0 and 0.25 g in the resistant checks.

Similarly, striga shoot height was significantly (P<0.01) reduced in the exotic varieties (P-9401, P-9403 and SRN-39) and the two local varieties (Ayefere-Asfachew and Wotere). While striga grew as tall as 33 to 35 cm in the susceptible checks, the striga shoot height in these varieties was only in the range of 2 to 15 cm.

Sorghum shoot weight, root weight and plant height

The results of this study indicated that the dry shoot weight and plant height of sorghum were significantly affected by striga infestation as compared with those of non-infested sorghum (Table 3). Many reports (Doggett, 1988; Ramaiah and Parker, 1982; Stewart et al., 1988) have indicated that striga infestation markedly reduces leaf, stem and head growth in sorghum. Similarly, in the present study striga infestation significantly (P<0.05) reduced sorghum shoot weight and plant height (Table 4). This was further verified by the negative significant correlation (r) values of –0.31 and –0.38 between striga count and sorghum shoot weight and plant height, respectively. Sorghum plants in striga-infested soil weighed on the average 54 g $plant^{-1}$ and grew as tall as 92 cm as compared to a weight of 66 g $plant^{-1}$ and 118 cm in height in striga-free soil. The shoot weight and plant height of the 15 varieties under striga infestation were reduced in the range of 3 to 47% and 0.6 to 51%, respectively. The highest reduction in height was recorded in Merare and the least in Ayefere-Asfachew and P-9401. These results are in accordance with the work of Gworgwor and Weber (1991) who also reported a reduction in plant height due to striga infestation in sorghum. According to Ramaiah and Parker (1982) and Stewart et al. (1988) the reduction in the growth and vigour of the host plant is attributed to many effects, such as the export of carbon to the parasite, the parasite-induced reduction of host photosynthesis and a change in growth regulator hormonal balance in the infested plants.

While reducing aboveground growth in the host plant, striga infestation was found to stimulate root growth (Igbinnosa and Okonkwo, 1991), though this was not observed in the present study, which indicated that striga infestation did not significantly affect root weight (Table 3).

Table 2

Striga count (shoots/pot), striga biomass weight (g/pot) and striga plant height (cm) in different sorghum varieties

Varieties	Total striga count*	Difference between total striga count and that of the resistant control	Striga biomass weight *	Difference between striga biomass weight and that of the resistant control	Striga shoot height	Difference between striga shoot height and that of the resistant control
SSGM 022	1.17b	1.05	0.98ab	0.98	29.8ab	27.8
SSGM 033	1.22b	1.10	0.70bc	0.70	23.0bc	21.0
SSGM 037	1.29ab	1.17	1.11ab	1.11	30.4ab	28.4
SSGM 050	1.23b	1.11	1.01ab	1.01	27.4ab	25.4
SSGM 063	1.23b	1.11	1.05ab	1.05	33.4ab	31.4
Kitign Ayefere	1.13b	1.01	0.91ab	0.91	22.6bc	20.6
Ayefere-Asfachew	0.71c	0.59	0.36cd	0.36	12.4c-e	10.4
P-9401	0.12d	–	0.0d	–	2.0e	–
P-9403	0.68c	0.56	0.25d	0.25	15.0cd	13.0
SRN 39	0.35d	0.23	0.0d	0.00	4.6de	2.6
Wotere	0.77c	0.65	0.36cd	0.36	14.8cd	12.8
Merare	1.42ab	1.30	1.38a	1.38	36.8a	34.8
Jigurti	1.30ab	1.18	0.97ab	0.97	27.6ab	25.6
Degalit	1.44ab	1.32	1.33a	1.33	33.4ab	31.4
Key # 8574	1.62a	1.50	1.37a	1.37	35.0ab	33.0
LSD (5%)	0.33		0.41		10.8	
CV (%)	25.3		41.2		36.9	

* Analyses were performed after logarithmic transformation of data [Log (X+1)]. Column values followed by the same letter (s) are not significantly different at the 5% level using Duncan's Multiple Range Test.

Table 3
Analysis of variance for the dry shoot weight (DSSWt), dry root weight (DSRWt) and plant height (SPH) of 15 sorghum genotypes grown in striga-infested and striga-free pots

Source	Mean squares			
	df	DSSWt	DSRWt	SPH
Striga treatment (St)	1	3055.504**	319.602NS	14951.111*
Error	2	39.524	33.881	498.411
Variety	14	1510.501***	450.394***	7485.925***
St × Variety	14	193.625NS	59.975NS	1029.230
Error	56	364.994	67.192	563.373
CV (%)		31.9	26.5	22.6

*,**,*** significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table 4
Dry shoot weight and plant height of sorghum varieties in striga-free and striga-infested pots

Varieties	Dry shoot weight (g/plant)			Plant height (cm)		
	With striga	Without striga	% reduction in infested plants	With striga	Without striga	% reduction in infested plants
SSGM 022	67	59	12	103	86	17
SSGM 033	93	66	29	140	106	24
SSGM 037	58	36	38	91	50	45
SSGM 050	64	62	3	102	97	5
SSGM 063	60	43	28	85	61	28
Kitign Ayefere	88	70	20	164	125	24
Ayefere-Asfachew	71	82	–13	160	159	0.6
P-9401	43	51	–16	74	72	3
P-9403	49	37	24	66	79	–16
SRN 39	64	40	38	69	72	–4
Wotere	93	87	6	169	148	12
Merare	51	44	14	170	84	51
Jigurti	65	54	17	173	121	30
Degalit	76	56	26	127	72	43
Key # 8574	45	24	47	76	49	36
Mean	66	54		118	92	
LSD (5%)	5.7			20.3		

Conclusion

The results of this study revealed that many of the local varieties claimed by farmers to be resistant to striga generally exhibited a disappointing level of resistance. However, two local varieties (Ayefere-Asfachew and Wotere) were of some interest. The number of striga supported, the striga biomass weight and the striga shoot height were significantly lower in these varieties. Moreover, the aboveground shoot weight and plant height of these varieties were least affected by striga infestation. This could be explained by some degree of resistance to the parasite. All striga-related parameters in these two varieties were statistically comparable to that recorded in the released resistant varieties (P-9401 and P-9403). Generally, these varieties would be of interest if good yield potential were associated with their low susceptibility to striga attack or if they had promise for research efforts to incorporate striga resistance into an agronomically elite background. Therefore, further field and more efficient laboratory testing must be conducted with these varieties to verify their reactions. This finding would not only help to show how *S. hermonthica* reduced the performance and yield of the different sorghum varieties which were praised for their resistance, but also assist farmers in choosing the best variety of sorghum to cultivate under striga infestation.

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PHYTOREMEDIATION OF CADMIUM-CONTAMINATED SOIL BY *BRASSICA* SPECIES

K. S. AHMED, B. S. PANWAR and S. P. GUPTA

DEPARTMENT OF SOIL SCIENCE, CCS, HARYANA AGRICULTURAL UNIVERSITY, HISAR, INDIA

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Phytoremediation is a green technology for the sustainable remediation of surface soils contaminated with toxic heavy metals. When added to soils the chelating agent ethylenediamine tetraacetic acid (EDTA) increased the solubility of heavy elements for plant uptake during phytoremediation. A greenhouse experiment was carried out with two *Brassica* species (*Brassica juncea* and *Brassica carinata*) grown on artificially contaminated soil (20 and 40 mg Cd kg^{-1}) with EDTA added at a rate of 1 g kg^{-1} soil. With increasing Cd (0, 20 and 40 mg Cd kg^{-1} soil) contamination the biomass of both the *Brassica* species decreased. However, *Brassica juncea* was more tolerant of high levels of Cd in the soil in comparison to *B. carinata*. The results indicated that EDTA made the cadmium more available to the plants and lowered the Cd content of the soil. The magnitude of the increase in tissue (stem, leaf and root) Cd concentration was higher in *B. juncea* than in *B. carinata* and after the application of chelating agent (EDTA). The *Brassica juncea* species of Indian mustard has better potential for the phytoremediation of soil heavily contaminated with Cd (40 mg Cd kg^{-1} soil).

Key words: phytoremediation, Cd uptake, chelating agent, *Brassica juncea*, *Brassica carinata*

Introduction

The soils in different parts of the world are showing signs of degradation and contamination, which is an alarming and catastrophic situation requiring urgent rejuvenation and decontamination. Agricultural soils generally receive most of their cadmium pollution from the atmosphere, fertilizers, pesticides and manure (Nriagu, 1990; McLaughlin et al., 1996). Among the anthropogenic sources, phosphate fertilizers have been considered as the primary source of Cd that may contaminate soils (Fergusson, 1990). Cadmium can accumulate in the food chain and present a threat to crop quality as well as human and animal health. Today's techniques and strategies for the sustainable clean-up of the environment from hazardous wastes and heavy metals include phytoremediation.

Phytoremediation is only a practical option for moderately polluted soils which can be decontaminated from toxic heavy metals (Cd, Hg, Pb and Ni) using hyperaccumulator plant species. This is a three-stage process involving the high uptake of heavy metals by the roots, transportation to the shoot and the sequestration of metal within the shoot. Phytoremediation using hyperaccumulator plants may provide an effective and *in situ* way of removing heavy metals from contaminated soils (Baker et al., 1994). Indian mustard

(*Brassica juncea)* accumulates high amounts of Pb, Cd, Cu, Ni and Zn in the shoots (Blaylock et al., 1997). The development of phytoremediation must be a process of matching the plants to the particular situation. High biomass crops such as *Brassica* species may be useful, especially with the addition of metal chelating or acidifying compounds which would boost the metal concentrations in the crops by desorbing the metals from the soils and overcoming any diffusional limitations to their transport to the site of uptake in the roots and translocation to the shoots. Metal uptake and accumulation in the shoots of *Brassica juncea* have been shown to be enhanced by the application of chelators. EDTA was the best chelating agent for mobilizing Pb from soil particles (Huang et al., 1997) by gradual acidification. The ability of this chelatant to bind metal ions in extremely stable complexes can be utilised not only for the desorption of sorbed ions, but for the dissolution of insoluble metal compounds as well. Before phytoremediation can be effectively exploited on contaminated soils, a better understanding of the metal uptake by hyperaccumulator species is necessary.

The present investigation was undertaken to study the phytoremediation of Cd-contaminated soil by *Brassica* species with and without the application of chelating agent (EDTA) to the soil.

Materials and methods

A pot experiment using sandy loam soil (*Typic Ustochrept*) was conducted in the greenhouse. Some selected characteristics of the soil are as follows: pH (1:2) 7.8; electric conductivity (E.C.) 0.38 dS m^{-1}; organic carbon content 0.41%; cation exchange capacity (C.E.C.) 12.70 cmole (p+) kg^{-1} soil; DTPA-extractable Cd 0.24 mg kg^{-1} soil. Five kg of air-dried soil (2 mm sieve) was filled into earthen pots which were lined with polyethylene to avoid contamination. The treatments consisted of three levels of Cd (0, 20 and 40 mg Cd kg^{-1} soil as cadmium chloride) and two levels of EDTA (0 and 1 g kg^{-1} soil as disodium salt) in all possible combinations. Prior to sowing, the treated pots were saturated with water and kept for one month to equilibrate the soil artificially contaminated with cadmium. *Brassica juncea* and *Brassica carinata*, two species of Indian mustard, were sown as test crops. The basal fertilizer requirement of the crop (50, 50, 62, 10, 10, 5 and 20 mg kg^{-1} soil of N, P, K, Mn, Fe, Zn and S, respectively) was added in solution form and mixed thoroughly with the soil before sowing. Each treatment was replicated three times in a completely randomized design. The pots were irrigated to field capacity with deionized water throughout the growth period. Ten seeds of *Brassica juncea* or *Brassica carinata* were sown in each pot and thinned to five plants after germination. EDTA was applied 6 weeks after crop germination at the rosette stage in solution form. Both the crops were harvested 8 weeks after germination (blooming stage) and washed with distilled water. The plant material was air dried, then oven dried at 60°C for 72 hours and weighed. Half a gram of processed plant tissue (ground to pass through a 425 µm sieve in a mill) was digested using 20 ml of a di-acid mixture of concentrated H_2SO_4 : $HClO_4$ (4:1) and then diluted to 25 ml with distilled water. The cadmium in the digested solution was determined using an atomic absorption spectrophotometer (Lindsay and Norvell, 1978).

Results and discussion

Visual toxicity symptoms

The yellowing of young leaves was observed one week after germination in pots treated with 40 mg Cd kg^{-1} soil in both the crops. As the growth period proceeded the crops began to turn green, but more stunted plant height was recorded in *B. carinata* than in *B. juncea*. After the addition of EDTA in solution form, the crops started wilting due to the boosting of the Cd concentration in plant tissue from the soil.

Dry biomass of plants

In the eight-week growth period *Brassica juncea* produced almost double the biomass of *Brassica carinata* in cadmium-contaminated soil. There was no adverse effect on crop growth of EDTA application alone. However, it decreased the biomass in both the genotypes at 20 and 40 mg Cd kg^{-1} soil level, which may be due to the increased solubility of Cd in the soil, resulting in high Cd accumulation in the plants. This reduced photosynthesis and caused an internal water deficit in the shoot system due to poor root development. The magnitude of the decrease in biomass (stem, leaf and root) with the increasing rate of Cd was higher in the presence of EDTA in both the genotypes.

Data on the influence of EDTA on the biomass of the stem, leaf and root (Fig. 1) averaged over the Cd levels, revealed that with the application of EDTA the stem yield of *Brassica juncea* decreased by 28% and that of *B. carinata* by 35%, showing that the yield reduction was greater for *B. carinata*. The decrease in leaf yield was 14% in *B. juncea* and 35% in *B. carinata*, while the decrease in root yield due to EDTA application was 30% in *B. juncea* and 18% in *B. carinata*. This showed that the translocation of Cd was less from the roots to the shoots in *B. juncea* than in *B. carinata*, resulting in a greater decrease in stem and leaf yield and a smaller decrease in root yield in *B. carinata* as compared to *B. juncea*.

The effect of Cd and EDTA on the biomass of stem, leaf and root of *Brassica* species, averaged over the genotypes, is presented in Figure 2. The mean stem yield at 0, 20 and 40 mg Cd kg^{-1} soil without EDTA application was 4.8, 4.1 and 3.8 g pot^{-1}, respectively, whereas the corresponding yield with the application of EDTA was 4.3, 2.6 and 2.0 g pot^{-1}, respectively.

This indicated that with the application of EDTA the yield decreased by 10, 36 and 47% at 0, 20 and 40 mg Cd kg^{-1} soil level. The mean leaf yield at 0, 20 and 40 mg Cd kg^{-1} soil was 7.2, 6.4 and 6.0 g pot^{-1}, respectively, without EDTA and 6.8, 4.7 and 3.5 g pot^{-1} with EDTA, showing a decrease of 5, 26 and 41%, respectively, after the application of EDTA. This indicated that EDTA enhanced the absorption of Cd by the plant roots, resulting in a reduction in biomass due to Cd toxicity. The mean root yield at 0, 20 and 40 mg Cd kg^{-1} soil was 3.0, 2.7 and 2.3 g pot^{-1}, respectively, without EDTA and 2.5, 1.9 and 1.6 g pot^{-1} with EDTA, representing a yield reduction of 16, 29 and 30%, respectively. Similar phytoextraction capacity of *Brassica* species for heavy metals was observed by Laszlo (1999) and Ebbs and Kochian (1997).

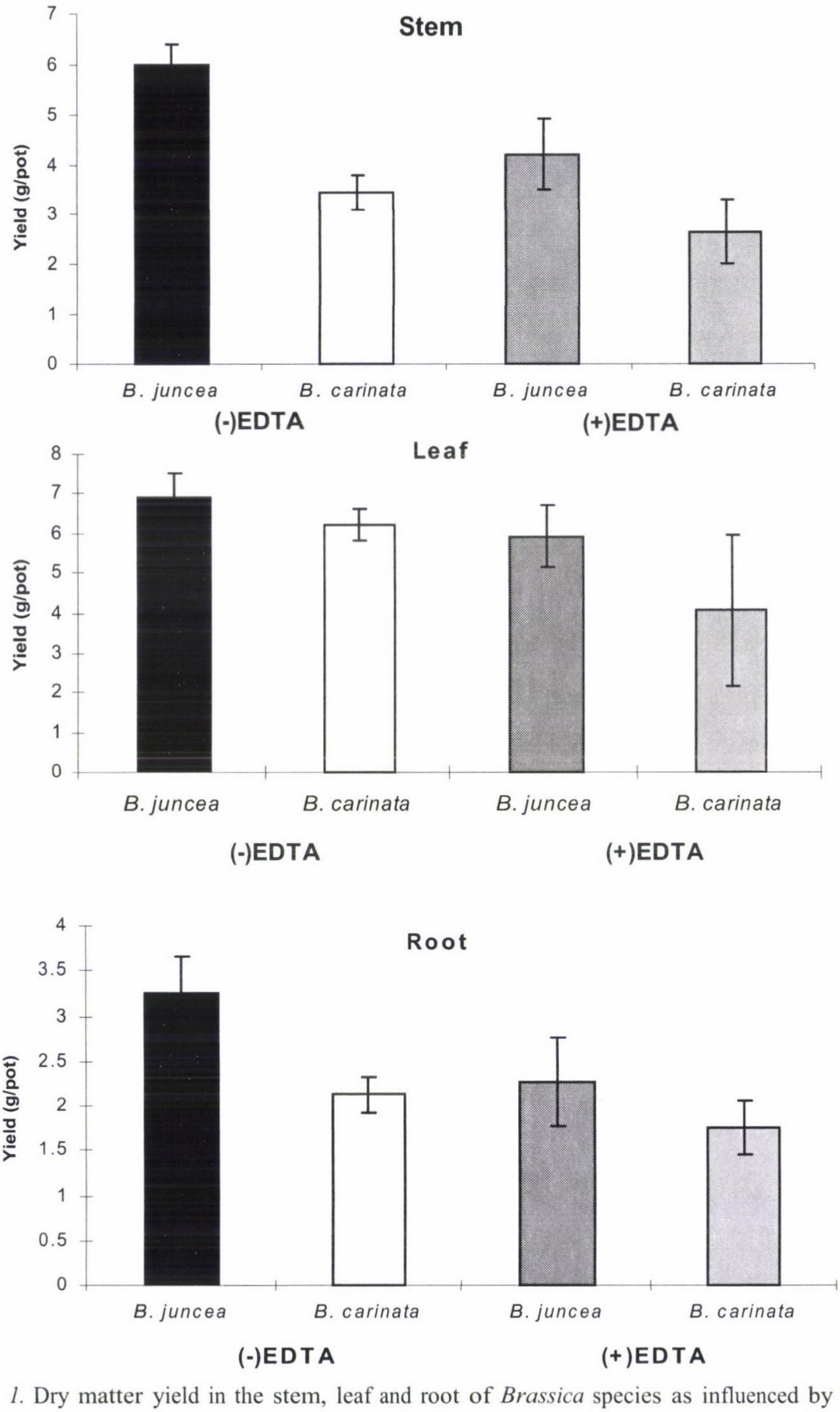

Fig. 1. Dry matter yield in the stem, leaf and root of *Brassica* species as influenced by EDTA, averaged over the Cd treatments

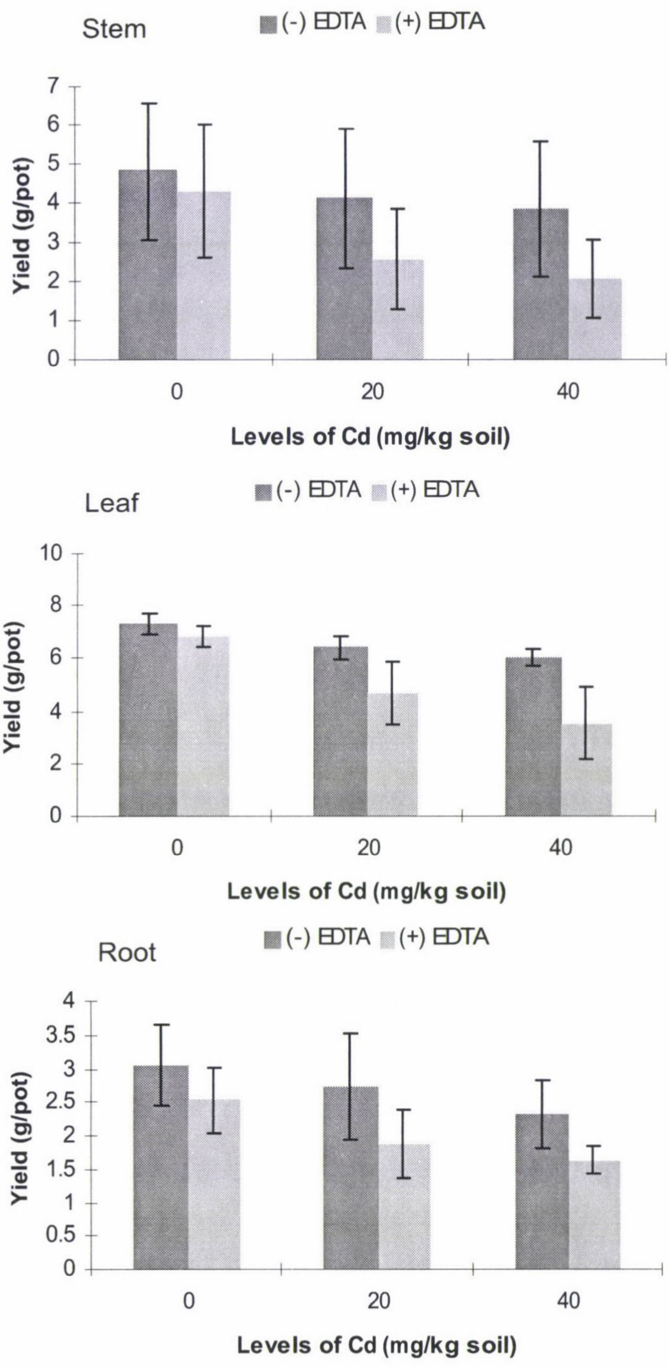

Fig. 2. Dry matter yield in the stem, leaf and root of *Brassica* as influenced by cadmium and EDTA, averaged over the *Brassica* species

Cadmium concentration in plants

Data on the influence of EDTA on the Cd concentration in the stem, leaf and root, averaged over the Cd levels (Fig. 3), revealed that the concentration of Cd in the stem of *B. juncea* increased by about 1.4 times and that of *B. carinata* by 2.3 times after the application of EDTA. In the leaf the Cd concentration increased by 1.4 times in *B. juncea* and 1.9 times in *B. carinata*. The Cd concentration in the root increased 1.5 times in *B. juncea* and 2.3 times in *B. carinata* after the application of EDTA. The magnitude of the increase in Cd concentration was thus greater for *B. carinata* than for *B. juncea*.

Figure 4 shows the effect of Cd and EDTA on the Cd concentration in the stem, leaf and root of *Brassica* species. At 0, 20 and 40 mg Cd kg^{-1} soil the mean Cd concentration in the stem without EDTA was 4.3, 12.6 and 28.5 μg g^{-1} dry matter, respectively, whereas the corresponding values with EDTA were 5.1, 22.5 and 54.2 μg g^{-1} dry matter. This shows a 1.2, 1.8 and 1.9 times increase in Cd concentration in the stem at 0, 20 and 40 mg Cd kg^{-1} after the application of EDTA. The mean leaf Cd concentration at 0, 20 and 40 mg Cd kg^{-1} soil was 6.0, 40.4 and 68.8 μg g^{-1} d.m., respectively, without EDTA and 6.7, 74.8 and 102.4 μg g^{-1} d.m. with EDTA. The increase in Cd concentration was 1.1, 1.9 and 1.5 times, respectively. The mean root Cd concentration at 0, 20 and 40 mg Cd kg^{-1} soil was 1.3, 35.9 and 53.9 μg g^{-1} d.m., respectively, without EDTA and 3.0, 70.0 and 94.1 μg g^{-1} d.m., respectively, with EDTA. The increase was 2.3, 2.0 and 1.7 times at 0, 20 and 40 mg Cd kg^{-1} soil after the application of EDTA averaged over the *Brassica* genotypes. A similar trend was recorded by Lambrecht et al. (1999) in Indian mustard.

Cadmium uptake by plants

The effect of EDTA on the Cd uptake by *Brassica* species, averaged over the Cd treatments, is illustrated in Figure 5a. In the absence of EDTA the Cd uptake in the aboveground organs (stem + leaf) in *B. juncea* was 407.8 μg pot^{-1}, whereas in EDTA-treated pots the Cd uptake was 456.6 μg pot^{-1}, indicating a 12% increase in Cd uptake. In the case of *B. carinata* the Cd uptake in the absence of EDTA was 202.8 μg pot^{-1}, whereas in EDTA-treated pots the Cd uptake was 185.3 μg pot^{-1}, showing a decrease of about 8% due to the poor growth of the crop because of Cd toxicity at high Cd concentrations.

The effect of Cd and EDTA on Cd uptake by *Brassica* averaged over the *Brassica* species is depicted in Figure 5b. The Cd uptake in the aboveground organs (stem + leaf) was 64.1, 312.9 and 583.9 μg pot^{-1} in the absence of EDTA at 0, 20 and 40 mg Cd kg^{-1} soil, respectively. The corresponding values with EDTA application were 67.6, 408.2 and 487.2 μg pot^{-1}, indicating an increase of 5 and 30% in Cd uptake at the 0 and 20 mg Cd kg^{-1} soil level. However, at 40 mg Cd kg^{-1} soil the Cd uptake decreased by 9%, which is mainly attributed to the poor growth and biomass of *B. carinata* at this Cd level in the presence of EDTA. The findings of this investigation confirm the results of Luo et al. (1999) and Kirkham (1999).

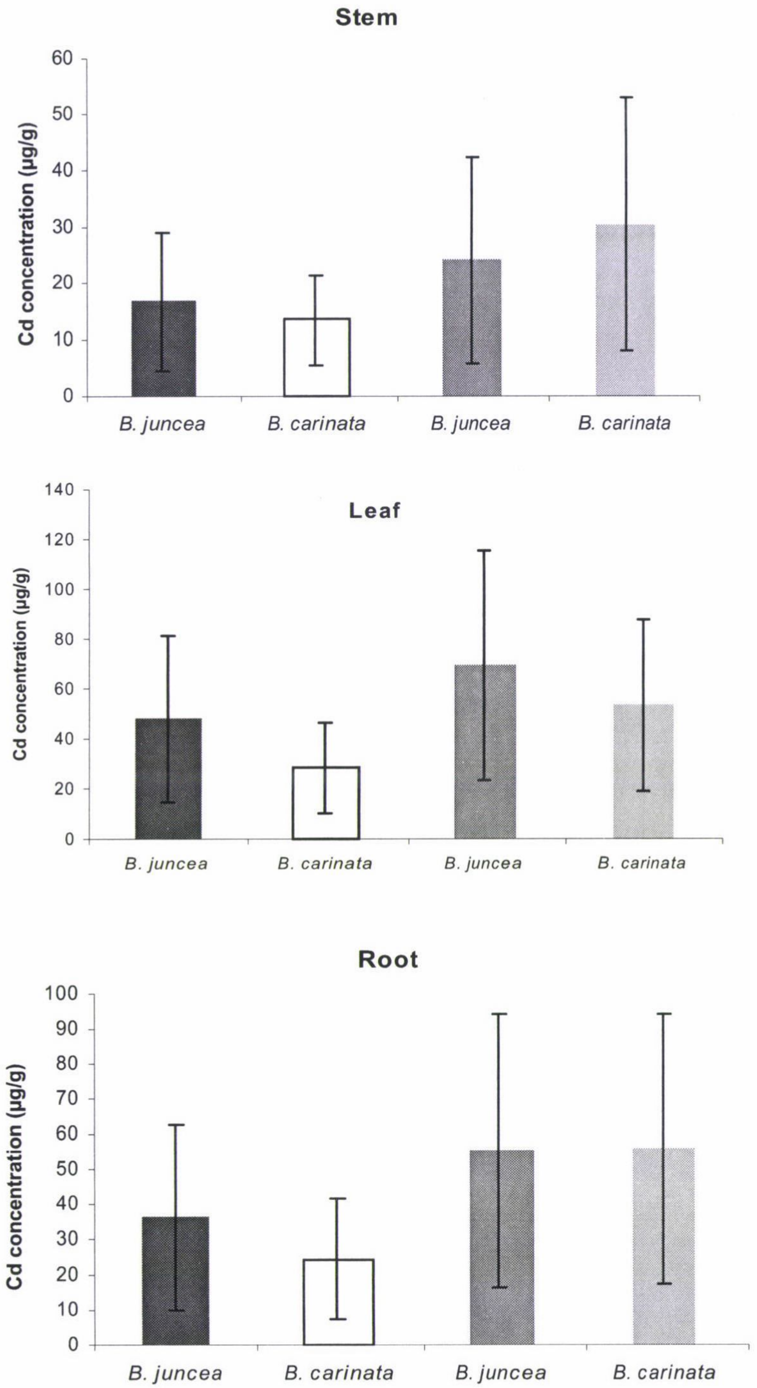

Fig. 3. Cadmium concentration in the stem, leaf and root of *Brassica* as influenced by EDTA, averaged over the Cd treatments

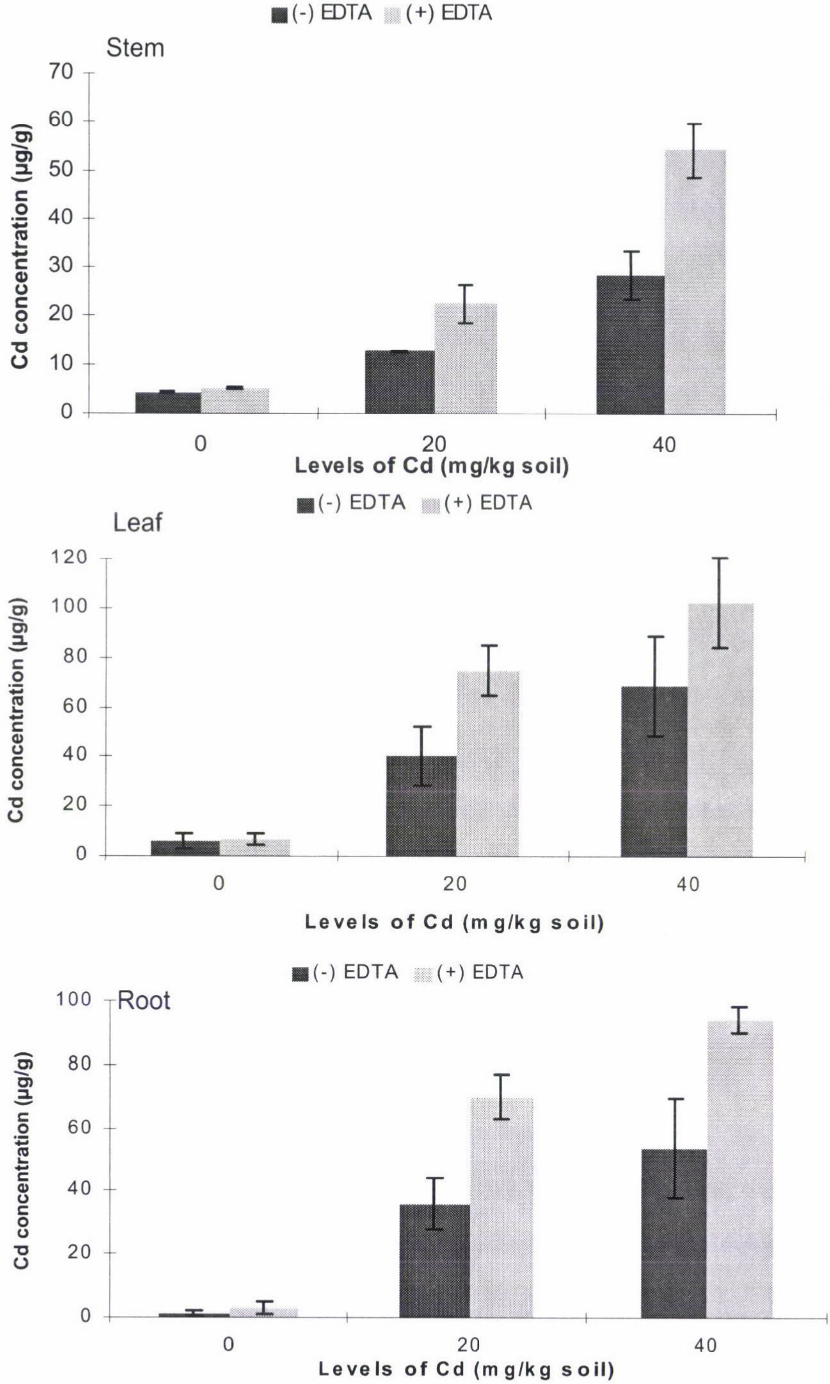

Fig. 4. Cadmium concentration in the stem, leaf and root of *Brassica* as influenced by cadmium and EDTA, averaged over the *Brassica* species

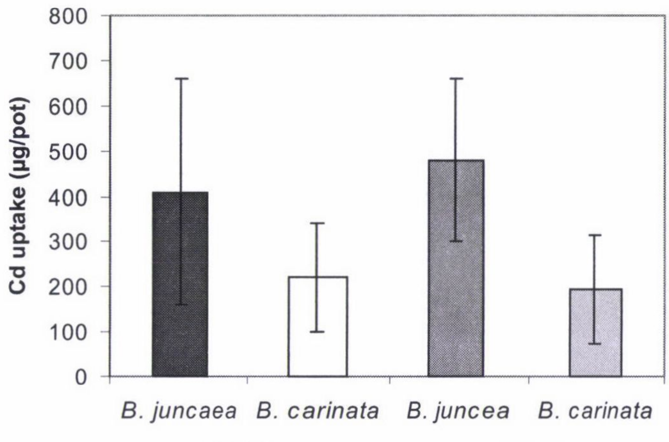

Fig. 5a. Cadmium uptake in aboveground organs (stem+leaf) of *Brassica* as influenced by EDTA, averaged over the Cd treatments

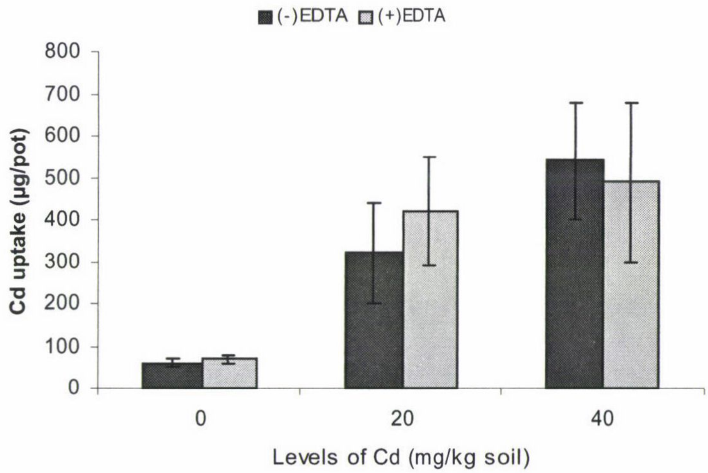

Fig. 5b. Cadmium uptake in aboveground organs (stem+leaf) of *Brassica* as influenced by EDTA, averaged over the *Brassica* species

Conclusions

Brassica species can grow well in soil freshly contaminated with 20 and 40 mg Cd kg^{-1} and have high potential for removing Cd from the soil in the course of phytoremediation. *Brassica juncea* performed better as a hyperaccumulator for cadmium, producing higher biomass than *B. carinata*. EDTA proved to be an effective chelating compound for mobilizing the metal from the soil and enhanced the Cd concentration and uptake in Indian mustard.

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HYBRID SEED PRODUCTION IN CASSAVA *(MANIHOT ESCULENTA* CRANTZ) AFTER NATURAL AND ARTIFICIAL POLLINATION IN A HUMID AGROECOLOGICAL ZONE

M. N. OGBURIA and K. OKELE

DEPARTMENT OF CROP/SOIL SCIENCE AND FORESTRY,
RIVERS STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY, PORT HARCOURT, NIGERIA

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An effective pollination system is an important pre-requisite for successful hybridization in any breeding programme. A field experiment was conducted between March 1998 and March 1999 at the Teaching and Research Farm of the Rivers State University of Science and Technology, Port Harcourt in a humid agroecological zone of Nigeria, to evaluate ten selected cassava clones for hybrid seed production efficiency after natural and artificial pollination.

The time to 50% flowering and the number of pistillate and staminate flowers showed significant variation ($P \geq 0.05$) in the study. More staminate than pistillate flowers were produced in all clones, at a ratio of 8:1= staminate: pistillate per clone.

Hybrid seed production was significantly different ($P \geq 0.05$) in the ten selected clones of cassava after natural and artificial pollination. Natural pollination was more effective as regards the rate of seed set (26.9 seeds on 0.056 ha^{-1}, equivalent to 480.9 seeds ha^{-1}) than artificial pollination, which produced 8.8 seeds on 0.056 ha^{-1} or a calculated equivalent of 156.3 seed ha^{-1}.

For optimal hybrid seed production, natural pollination using male sterile females and desirable male fecund parents, well arranged in the field to encourage effective natural cross-pollination, either by wind or insects, is suggested for increased hybrid seed production in a cassava breeding programme in a humid ecological zone of Nigeria.

Key words: pollination, hybrid seeds, hybridization, breeding, natural pollination, artificial pollination

Introduction

Cassava (*Manihot esculenta* Crantz) is a shrubby perennial, which belongs to the family *Euphorbiaceae.* It is grown extensively as one of the most important staple food crops in tropical regions of the word (Hahn et al., 1990). It is a vegetatively propagated monoecious crop, but sexual propagation frequently occurs through seed (Hahn et al., 1990), and this provides a useful tool for plant improvement.

Cassava exhibits both cross-pollination and self-pollination in nature. The proportion of cross-pollination depends on the flowering habit of the genotypes and the physical arrangement of the population (CIAT, 1975). Open-pollinated flowers produce both self- and cross-pollinated seeds in proportions which depend on genotype, planting design and the type of pollinating insects present (Hershey, 1981). However, the production of large quantities of seed is laborious and costly, because the rate of abortion becomes more pronounced once pollination is done manually (Kawano, 1980; Hahn, 1982; Nassar, 1989). The fertility rates of different cultivars of cassava after pollination are variable and have been observed to be very low (Hershey, 1981). It has been discovered that, for controlled pollination, an average of about one seed per pollination is commonly achieved from a maximum of three in the trilocular ovary. The genotype of the female appears to be more important in determining success than that of the pollen source (Hershey, 1981).

From the literature, it is evident that no work has been reported on the seed set potentials of locally or internationally synthesized hybrids in this environment. Efforts to increase seed set potentials in cassava by means of heat shock as a physiological treatment which yielded tangible results, with twice the number of ovules per trilocular pistil of cassava, were reported recently by Ogburia et al. (2000). Increased hybrid seed production is an important and integral aspect of cassava breeding, the improvement of which requires concerted effort even though some of the sexual reproductive barriers have been found to be of embryological or cytogenetic origin (Ogburia and Adachi, 1994).

The formulation of an appropriate hybridization strategy could surmount some of the outlined reproductive problems, and an effective pollination system could lead to the development of new cassava cultivars with combined traits of high yield, wider ecological adaptation, a reasonable level of protein content and resistance to major diseases and pests (CIAT, 1974, 1975 and 1976). Therefore, this research was conducted to evaluate different cassava cultivars for hybrid seed set efficiency after natural and artificial pollination in a humid agroecological zone of Nigeria.

Materials and methods

This experiment was conducted over a duration of twelve calendar months (March, 1998–March, 1999) spanning two cropping seasons (both dry and wet seasons) with one planting. The experimental location was the Teaching and Research Farm of the Rivers State University of Science and Technology, Port Harcourt, in the humid agroecological zone of Nigeria. The top 15 cm of soil, a Typic Paleudult, before minimum tillage, had a pH of 4.7, 1.60% organic matter, 0.08% total N, 42 ppm Bray's P1 and 1.63, 1.44, 0.33 me/100 g exchangeable K, Ca and Mg, respectively.

Ten selected cultivars of cassava representing different genotypes were used in a randomized complete block design (RCBD). Nine of the cultivars were improved varieties from the Tropical *Manihot* series (TMS) TMS 4488, TMS 30572, TMS 4(2)1425, TMS 91/00153, TMS 84/00275, TMS 82/00058, TMS 87/00611, TMS 30555 and TMS 91/0006, obtained from the International Institute of Tropical Agriculture (IITA), Onne substation, Rivers State, while one was a local cultivar (Wocha) obtained from the Rivers State University of Science and Technology Teaching and Research Farm, Port Harcourt.

Eight cassava cuttings (each measuring 25 cm in length) from each of the ten different clones were planted in one plot per clone in each block and replicated four times, at random, giving forty experimental units for the entire experimental set-up. The experimental area was 560 cm^2 (0.056 ha), which was divided into forty units (plots) each measuring 2 m × 3 m with 1.5 m separating the plots. The distance between blocks (replications) was 2 m. All ten cassava cultivars were planted using a slanting method at an angle of 45°–60°, with 1 m × 2 m spacing to give 5,000 plants ha^{-1}. All the cuttings were planted at the same time in March, 1998.

The plots were left untreated with any form of agrochemical throughout the duration of the experiment. Hoeing and hand pulling at the 4th, 12th, 20th and 28th weeks controlled weeds after planting, giving a total of 4 weedings.

Time to 50% flowering was recorded for each cultivar between the 14^{th} and 24^{th} weeks after planting (WAP) in all the flowering cultivars to determine differences in the time of flowering between the cultivars under investigation. The number of staminate and pistillate flowers was counted using five representative plant stands per plot to determine the staminate to pistillate flowering ratio for each cultivar. Artificial pollination (AP) and sampling were carried out in October–November, 1998, towards the end of the rainy season. Artificial pollination took place at anthesis in both staminate and pistillate flowers as described by Kawano (1980) and Hahn (1982). Open pollinated (OP) flowers of the same age as the artificially pollinated (AP) ones were randomly sampled together and evaluated for the number of filled ovaries in the field.

Matured fruits from open pollination (OP) and artificial pollination (AP) were harvested in December, 1998 and through January, 1999, as described by Biggs et al. (1986). Seed flotation in water, and the weight and size (length) of the seed clones were determined as described by Biggs et al. (1986).

Results and discussion

There was significant variation at the $p \geq 0.05$ level (DMRT) in the time of flowering among the flowering cultivars of cassava investigated (Table 1). The local cultivar did not flower till the time of harvest 52 weeks after planting (WAP), while two of the improved cultivars (TMS 92/00153 and TMS 82/00058) produced a negligible number of flowers. TMS 30572 flowered within 15 WAP, while TMS 91/000153 took the longest period (24 WAP) up to 50% flowering. Cassava produces more staminate than pistillate flowers. The average flowering ratio for all the genotypes was 8:1 staminate to pistillate flowers. This observation confirms that of Kawano (1980), who found that male flowers usually outnumbered the female flowers available for pollination, which is a limiting factor for the mass production of hybrid seed. The rate of flower abortion and fertilization differed significantly for the ten cassava genotypes (Tables 2 and 3). Open pollination (OP) exhibited lower floral abortion (65.8%) than artificial pollination (90%). On the other hand, open pollination (OP) produced more filled (fertilized) ovaries (30.1%) than artificial pollination (AP) (9.6%). The rate of flower abortion of cassava plants became high when they were not allowed to carry out pollination by natural means (insects or wind). This result tallies with that of Nassar (1989), who reported that natural pollination was more effective in producing hybrid seeds than manual pollination in Brazil.

The seed production and physical seed analysis of the ten cassava cultivars are shown in Tables 4 and 5. The number of seeds per ovary showed significant differences ($P \geq 0.05$) among the ten cassava cultivars after both OP and AP. Generally, the average number of seeds per trilocular ovary ranged from 1–3 seeds per ovary in both OP and AP (Table 4).

Table 1
Flowering behaviour of selected cassava

Cassava clones	Time to 50% flowering (WAP)	No. of flowers/clone		S : P ratio
		Staminate	Pistillate	
TMS 2/00058	22.5a	0.00e	0.00d	0.00:0.00
TMS 4/00275	21.13a	102.5ab	4.25c	24.12:1
TMS 30572	14.75b	48.8d	11.25b	24.12:1
TMS 4 (2) 1425	16.13ab	51.7d	6.63bc	7.80:1
TMS 87/00611	19.00ab	88.7d	11.50b	7.72:1
TMS 4488	18.075ab	87.8bc	3.63bc	24.10:1
TMS 91/000153	23.50a	0.00e	0.00d	0.00:0.00
TMS 30555	16.50ab	11.8.a	18.63a	6.35:1
TMS91/00061	16.50ab	44.8d	7.00bc	6.40:1
Local cultivar	0.00c	00.0e	0.00d	0.00:0.00
CM	16.88	54.24	6.27	8.08:0.7

Means carrying the same letters in the same column do not significantly differ (DMRT 0.005≤P). Cultivars with 0.00 = non-flowering/negligible flowering. CM = cultivar mean, WAP = Weeks after planting.; S : P= staminate : pistillate

Table 2
Rate of floral abortion (abscission)

Cassava clones	No. of aborted flowers (% clone^{-1} 0.056 ha^{-1})	
	OP	AP
TMS 84/00275	95.0ab	100a
TMS 82/0058	–	–
TMS 30572	52.5de	87.5cd
TMS 4 (2) 1425	78.8a	95.0ab
TMS 87/00611	35.def	85.00ab
TMS 4488	73.8bcd	91.2bc
TMS 91/00153	–	–
TMS 30555	25.0f	75.0e
TMS 91/00061	100a	100e
Local cultivar	–	–
CM	60.60B	90.45A

Only means carrying different letters in the same column differ significantly DMRT (p≤0.05). Cultivars with a dash (–) signify non-flowering; OP = open pollination; AP = artificial pollination; CM = cultivar means.

The number of seeds per clone in cultivars which both flowered and were pollinated also showed a statistical difference (P≥0.05), which entirely depended upon the number of filled ovaries. Consequently, the quantity of seeds produced per method of pollination took the order OP>AP. TMS 30555 produced the highest number of seeds followed by TMS 30575, while the lowest number of seeds was recorded in TMS 4 (2) 1425.

Table 3
Number of filled ovaries (%)

Cassava clones	No. of aborted flowers (% clone^{-1} 0.056 ha^{-1})	
	OP	AP
TMS 84/00275	5.0c	0.0
TMS 82/0058	–	–
TMS 30572	47.5b	12.5bc
TMS 4 (2) 1425	21.3c	6.3c
TMS 87/00611	38.8b	15.0b
TMS 4488	23.3bc	8.8bc
TMS 91/00153	–	–
TMS 30555	75.0a	25.0a
TMS 91/0061	0.0	0.0
Wocha	–	–
CM	30.11A	9.64B

Means carrying different letters in the same column differ significantly DMRT (P>0.05).
Cultivars with a dash (–) signify non-flowering; cultivars with 0.0 means in OP and AP denote 100% floral abortion, hence no filled ovaries; OP = open pollination; AP = artificial pollination; CM = cultivars means.

Table 4
Seed production after open pollination (OP) and artificial pollination (AP)

Cassava clones	No. of seeds per ovary		No. of seeds per clone	
	OP	AP	OP	AP
TMS 84/00275	0.00	0.00	0.00	0.00
TMS 82/00058	–	–	–	–
TMS 30572	2.00	1.75	43.50ab	9.75b
TMS 4 (2) 1425	1.20	0.89	12.00b	4.25b
TMS 87/00611	2.90	2.06	40.63ab	10.00b
TMS 4488	1.90	1.59	26.88b	5.88b
TMS 91/00153	–	–	–	–
TMS 30555	2.30	2.43	65.50a	31.38a
TMS 91/00061	0.00	0.00	0.00	0.00
Wocha	–	–	–	–
CM	1.53	1.25	26.93	8.75
LSD (0.05≥P)	NS	NS	31.38	12.06

Means with different letters in the same column differ significantly, using LSD (0.05≥P). Cultivars with a dash (–) denote non-flowering, while those with 0.00 means were cultivars that exhibited 100% flower abortion. NS = non-significant

The average size (length in mm) of the seeds did not show any significant difference (P≤0.05) between the cultivars for either OP or AP flowers (Table 5). There was also a significant difference between the cultivars for the number of seeds that sank in water after OP and AP (Table 5). This variability could be attributed to differences in genotypes and may include genotype × environment interaction. The occurrence of seedless fruits from AP flowers, which was also occasionally observed in OP flowers, is consistent with the results of Chandraratna and Nanayakkara (1948) and Ogburia and Adachi (1995) who reported that hand pollution often resulted in seedless fruits, which seldom occurred after open pollination.

Table 5
Physical seed analysis

Cassava clones	Weight of seed*		Size of seed[+]		Floated		Sank	
	OP	AP	OP	AP	OP	AP	OP	AP
TMS 305555	9.6a	3.66a	5.4	5.41	9.88a	6.25	56.89a	22.13a
TMS 30572	5.4b	1.18c	4.9	4.13	9.50a	4.13	33.63b	5.63b
TMS 4 (2) 1425	2.2d	0.59d	1.8	2.16	2.75b	0.88	9.25b	3.13b
TMS 91/00153	–	–	–	–	–	–	–	–
TMS 84/00275	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TMS 84/0058	–	–		–	–	–	–	–
TMS 87/00611	3.9c	2.29b	5.4	6.36	8.50ab	3.13	3.38b	0.25b
TMS 87/4488	2.7d	1.09c	4.1	5.01	5.38ab	2.25	20.38b	2.63b
TMS 91/00061	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wocha	–	–	–	–	–	–	–	–
cm	3.40	1.26	3.09	3.30	5.14	2.38	20.50	5.68
S.E(±)	3.36	1.32	2.44	2.6	4.31	2.32	20.30	7.65
LSD (0.05>P)	0.66	0.24	NS	NS	6.9	NS	27.34	13.73

*: g; [+]: length in mm. Means with different letters in the same column differ significantly, using LSD (0.05≤P). Cultivars with a dash (–) denote non-flowering, while those with 0.00 means were cultivars with 100% flower abortion. OP = open pollination; AP = artificial pollination; NS = non-significant

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EFFECT OF WHEAT, LEGUME AND LEGUME-ENRICHED WHEAT RESIDUES ON THE PRODUCTIVITY AND NITROGEN UPTAKE OF RICE-WHEAT CROPPING SYSTEM AND SOIL FERTILITY

S. N. SHARMA and R. PRASAD

DIVISION OF AGRONOMY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI, INDIA

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Field experiments were conducted for two crop years at the Indian Agricultural Research Institute, New Delhi to study the effect of enriching wheat residue with legume residue on the productivity and nitrogen uptake of a rice-wheat cropping system and soil fertility. The incorporation of wheat residue had an adverse effect on the productivity of the rice-wheat cropping system. When it was incorporated along with *Sesbania* green manure, not only did its adverse effect disappear but the response to fertilizer N was also increased. There was no response to fertilizer N when *Sesbania* green manure was incorporated. When wheat residue was incorporated along with *Sesbania* green manuring, rice responded significantly to fertilizer N up to 120 kg N ha^{-1} in the first year and to 60 kg N ha^{-1} in the second year and at these levels of N, *Sesbania* + wheat residue gave 0.8 to 1.2 t ha^{-1} more grain, 0.6–1.0 t ha^{-1} more straw and 8–15 kg ha^{-1} more N uptake of rice resulting in 0.04–0.17% more organic C, 3–8 kg ha^{-1} more available P and 17–25 kg ha^{-1} more available K content in the soil than wheat residue alone at the same rates of N application. The respective increaseas caused by *Sesbania* green manure + wheat residue over *Sesbania* green manure alone were 0.3–0.5 t ha^{-1} in the grain and straw yield, 1–9 kg ha^{-1} in the N uptake of rice, 0.02–0.10% in organic C, 1–8 kg ha^{-1} in available P and 35–70 kg ha^{-1} in available K content in the soil. These treatments also gave higher residual effects in succeeding wheat than wheat residue alone. The incorporation of residues of both wheat and *Sesbania* is thus recommended to eliminate the adverse effect of wheat residue and to increase the beneficial effects of *Sesbania* green manuring.

Key words: *Sesbania* green manuring, mungbean residue, wheat residue, productivity, organic C, Kjeldahl-N, available P, available K

Introduction

Rice-wheat cropping systems occupy about 22 million hectares in Southern Asia and are the backbone of food security in several countries in this region (Fujisaka et al., 1994). This is, however, a highly intensive cropping system and each metric ton of grain harvested leads to the removal of 48 kg ha^{-1} major plant nutrients (N+P+K) (Gangiah and Prasad, 1999). Such large demands for plant nutrients in crops of rice and wheat, which have a duration of 4–5 months each, could not be met by organic manures and the farmers have almost completely shifted to chemical fertilizers, of which urea, diammonium phosphate and muriate of potash are the most popular. This use of high analysis fertilizers (containing high amounts of N, P and K only) as compared to ordinary superphosphate, which contains micronutrients as impurities, has created deficiencies of Zn and Fe (Takkar, 1996) and the soils are showing signs of fatigue, as judged by the decline in the yields of rice and/or wheat (Yadav et al., 1998) as well as a lower response to applied chemical fertilizer (Yadav, 1998). As a result of this the farmers are applying more and more fertilizer, which is creating problems of groundwater contamination with nitrates (Singh et al., 1995).

To overcome this problem integrated plant nutrient management involving organic manures/crop residues is receiving attention. However, due to ever increasing food demands in the region caused by a fast increase in the human population, the area under forage and fodder crops has declined and this has resulted in a reduced number of draught animals on the farms, which were the main source of energy. This decline in the farm animal population has been further accentuated by increased mechanization. The ultimate result is the decline in animal or farmyard manure (FYM). Attempts are therefore being made to generate organic residues *in situ* as green manure or dual purpose grain legumes (John et al., 1989; Sharma and Prasad, 1999). Attempts have also been made to incorporate rice/wheat straw (Prasad et al., 1999). However, rice/wheat straws have a higher C:N ratio (80 or above) and immobilize soil and applied N. On the other hand, legume residues have a desirable C:N ratio (15–25) for the mineralization of organic N. An attempt was therefore made to study the effect of lowering the C:N ratio by enriching wheat residue with legume residue on the productivity and N uptake of the rice-wheat cropping system and the chemical properties of the soil.

Materials and methods

Site and soil

A field experiment was conducted during 2 crop years (1995–96 and 1996–97) at the Indian Agricultural Research Institute, New Delhi (28° 38' N latitude; 77° 11' E longitude). The crop year in India starts with the onset of the monsoon in July and ends in the June of the succeeding year; there are two crop growing seasons, namely, *kharif* (July–Nov.) when rice is grown and *rabi* (Nov.–April) when wheat is grown. The soil of the experimental field was a sandy clay loam Fluvent having 51.1% sand, 23.7% silt and 25.2% clay, pH 8.2 (1:2.5 soil to water ratio), 0.75% organic C, 319 kg ha^{-1} alkaline permanganate-hydrolysable N, 15.0 kg ha^{-1} 0.5 M $NaHCO_3$-extractable P and 385 kg ha^{-1} 1 N NH_4OAc-extractable K, determined using the procedures described by Prasad (1998). A rice-wheat cropping system had been practised in this field for the last 10 years.

The experimental design was a split plot with three replications. The main plot treatments consisted of (i) Fallow, (ii) Fallow + Wheat residue, (iii) *Sesbania aculeata* L. grown to flowering and then incorporated as a green manure (SGM), (iv) SGM + wheat residue, (v) Mungbean (variety PS-6) grown to maturity with pods harvested and remaining crop residue incorporated during land preparation for rice (MRI) and (iv) MRI + wheat residue. The sub-plots were three N rates (0, 60 and 120 kg N ha^{-1}) applied to rice as urea.

Field techniques

The summer crops (main plot treatments) were seeded at a uniform row spacing of 30 cm on April 24 in 1995 and April 20 in 1996. Each summer crop received a basal dose of 20 kg N ha^{-1} as urea and 17 kg P ha^{-1} as ordinary superphosphate. The fallow plots received no fertilizer. After sowing the summer crops, wheat residue at a rate of 6 t ha^{-1} as per treatment (readily available from the wheat harvest in mid-April) was spread on the soil surface and left as mulch during the summer months. *Sesbania* green manure and mungbean residue with or without wheat residue was incorporated in the soil 10 days before preparing the land for rice, which is generally done with a puddler in a flooded field. Just before the final puddling, 22 kg P ha^{-1} as ordinary superphosphate, 33 kg K ha^{-1} as muriate of potash and 4.5 kg Zn ha^{-1} as zinc sulphate heptahydrate were applied to each plot. Nitrogen as urea as per treatment was applied in two splits, with half the dose 10 days after transplanting (DAT) and the rest at 30 DAT.

The rice was transplanted in mid-July with 2 to 3 seedlings of 21–25 days of age $hill^{-1}$ at a spacing of 20 × 10 cm. The rice was harvested on November 7 in 1995 and November 10 in 1996. The field was irrigated after the rice harvest and when the soil came to condition, the land was prepared by disking and planking. Wheat was sown in the third week of November and harvested in the second week of April. The wheat crop received a basal dose of 40 kg N ha^{-1} as urea, 22 kg P ha^{-1} as ordinary superphosphate and 33 kg K ha^{-1} as muriate of potash at the time of sowing.

Sampling and chemical analysis

Immediately prior to incorporation, plants from 1 m^2 of *Sesbania* and mungbean were cut at ground level, dried, weighed and finely ground for Kjeldahl N analysis (Prasad, 1998). At rice and wheat maturity, the grain and straw were harvested separately from each sub-plot (6 m^2 area). The grain yield was recorded at 140 g water kg^{-1} fresh weight, and the straw yield was expressed on an oven dry weight basis. The dried grain and straw samples were analysed for Kjeldahl N. Soil samples collected from 0–25 cm soil depth after completing each cycle of rice-wheat cropping were air dried to a constant weight, ground and sieved through a 6 mm sieve. They were analysed for organic C by Walkley and Black's (1934) method and for Kjeldahl N, 0.5 M $NaHCO_3^-$-extractable P and 1 N NH_4Oac-extractable K as per the procedure described by Prasad (1998).

Results and discussion

Summer legumes

Sesbania aculeata produced 5.3–5.5 t ha^{-1} dry matter and accumulated 130–138 kg N ha^{-1}, all of which was returned to the soil when the aboveground residue was incorporated into the soil as green manure. Mungbean produced 0.5–0.6 t ha^{-1} grain and 3.2–3.8 t ha^{-1} residue and accumulated 97–118 kg Kjeldahl N ha^{-1}, 15–21% of which was in the grain and the remaining 79–83% in the residue. Thus the incorporation of mungbean residue resulted in the recycling of 77–99 kg Kjeldahl N.

Grain yield

The incorporation of wheat residue had an adverse effect on the grain yield of rice at all levels of N in the first year of the study. In the second year of the study the adverse effects of wheat residue incorporation were mitigated at 120 kg N ha^{-1}, indicating the need for adequate N fertilization when wheat residue was incorporated (Table 1). These results differ from those reported by Prasad et al. (1999) who reported a significant increase in rice yield due to the incorporation of wheat residue. However, in their studies they also reported a reduction in the yield of wheat due to rice residue incorporation in the first year of study.

Table 1

Effect of nitrogen (N) on grain yield (t ha^{-1}) of rice and succeeding wheat as influenced by wheat, legume and legume-enriched wheat residue

N ($kg\ ha^{-1}$)	1995							1996						
	Fallow		*Sesbania*		Mungbean		Mean	Fallow		*Sesbania*		Mungbean		Mean
	C	WR	C	WR	C	WR		C	WR	C	WR	C	WR	
							Rice							
0	5.0	4.9	5.7	5.9	5.1	5.5	5.3	4.4	4.1	5.2	5.2	4.9	5.0	4.8
60	5.4	5.0	5.8	6.2	5.9	5.7	5.7	5.1	4.8	5.3	5.6	5.6	5.4	5.3
120	5.8	5.4	6.1	6.6	6.2	6.3	6.1	5.2	5.5	5.4	5.5	5.2	5.3	5.3
Mean	5.4	5.1	5.9	6.2	5.7	5.9	-	4.9	4.8	5.3	5.4	5.2	5.3	-
$L.S.D_{0.05}$														
R				0.6							0.3			
N				0.3							0.3			
R×N				0.5							0.5			
							Wheat							
0	3.4	3.7	4.5	4.1	4.8	4.7	4.2	3.8	4.0	4.3	4.3	4.3	4.6	4.2
60	3.6	4.0	4.5	4.7	4.8	4.8	4.4	4.3	4.5	4.6	4.4	4.9	4.7	4.6
120	4.2	4.4	4.0	4.4	4.7	4.9	4.5	4.5	4.6	4.7	4.9	4.8	4.7	4.7
Mean	3.7	4.1	4.3	4.4	4.8	4.8	-	4.2	4.4	4.5	4.6	4.7	4.6	-
$L.S.D_{0.05}$														
R				0.6							0.3			
N				0.3							0.3			
R×N				0.6							0.6			

C: control, WR: wheat residue, R: residue, N: nitrogen

When wheat residue was incorporated along with *Sesbania* green manure or mungbean residue, not only did its adverse effect disappear but the response to fertilizer N also increased. There was no significant response to fertilizer N in *Sesbania* green manured plots without wheat residue. When wheat residue was also incorporated with *Sesbania* green manure, the grain yield of rice increased significantly with an increase in the rate of nitrogen application up to 120 kg N ha^{-1} in 1995 and 60 kg N ha^{-1} in 1996. The results of the present study thus show that the enrichment of wheat residue with legume, *Sesbania*/mungbean residue resulted in greater rice productivity than the incorporation of legume residue alone and overcame the adverse effect of wheat residue incorporation. The results with legume residues are in conformity with those reported earlier (Sharma et al., 1995; Sharma and Prasad, 1999). The advantage of *Sesbania*/mungbean green manuring was also reported by Misra and Prasad (2000).

Mungbean residue incorporation before rice showed the highest residual effect and increased the grain yield of succeeding wheat over fallow by 0.5–1.4 t ha^{-1} without N application to preceding rice, 0.6–1.2 t ha^{-1} with 60 kg N ha^{-1} and 0.3–0.5 t ha^{-1} with 120 kg N ha^{-1}. *Sesbania* green manure, which increased the rice yield more than mungbean residue, had a significant residual effect only at the lower level of N application to rice. *Sesbania* residue probably decomposed faster than mungbean residue at the higher rate of N application and thus had no residual effect on the succeeding crop. Wheat residue incorporation with or without *Sesbania* green manure or mungbean residue showed no residual effect on the grain yield of succeeding wheat.

Straw yield

Wheat residue had no significant effect on the straw yield of rice when it was incorporated without legume residues (Table 2). When wheat residue was incorporated with *Sesbania* green manure and mungbean residue it increased the straw yield of rice by 0.9 and 0.6–1.1 t ha^{-1}, respectively, over fallow. The increases in the straw yield of rice with *Sesbania* green manure and mungbean residue alone over fallow were 0.5–0.7 and 0.4–0.5 t ha^{-1}, respectively. Thus, wheat residue showed some advantage when it was incorporated with legume residues.

Sesbania green manuring increased the straw yield of succeeding wheat over fallow by 0.7–1.0 t ha^{-1} without N application, 0.4–1.1 t ha^{-1} with 60 kg N ha^{-1} and 0.5–0.6 t ha^{-1} with 120 kg N ha^{-1}; the respective increases with mungbean residue were 0.8–1.4, 0.8–1.5 and 0.5–1.2 t ha^{-1}. Thus, fertilizer N had a priming effect and decreased the residual effect of *Sesbania* green manuring and mungbean residue incorporation. The incorporation of wheat residue had no significant residual effect on the straw yield of succeeding wheat.

As regards the effect of N applied to rice, the straw yield of rice increased significantly when the rate of N application was increased from 0 to 60 kg N ha^{-1} in 1995 and from 0 to 120 kg N ha^{-1} in 1996, whereas the straw yield of succeeding wheat increased significantly only when the rate of N application to rice was increased from 0 to 120 kg N ha^{-1} in both years.

Table 2

Effect of nitrogen on straw yield (t ha^{-1}) of rice and succeeding wheat as influenced by wheat, legume and legume-enriched wheat residue

N (kg ha^{-1})	1995							1996						
	Fallow		*Sesbania*		Mungbean		Mean	Fallow		*Sesbania*		Mungbean		Mean
	C	WR	C	WR	C	WR		C	WR	C	WR	C	WR	
							Rice							
0	4.6	4.8	5.4	5.7	5.0	5.4	5.1	6.3	6.1	6.9	7.3	6.8	6.5	6.6
60	5.2	5.1	5.6	6.0	5.6	5.6	5.5	6.8	6.9	7.2	7.5	7.2	8.6	7.5
120	5.4	5.2	5.7	6.2	5.8	6.1	5.7	7.1	8.5	8.3	8.2	7.7	8.6	8.1
Mean	5.1	5.0	5.6	6.0	5.5	5.7	-	6.8	7.2	7.5	7.7	7.3	7.9	-
L.S.D$_{0.05}$														
R				0.5							0.9			
N				0.4							1.0			
R×N				0.6							1.8			
							Wheat							
0	4.3	4.5	5.3	5.5	5.7	5.4	5.1	4.5	4.6	5.2	5.4	5.3	5.5	5.1
60	4.6	4.7	5.7	5.5	6.1	5.9	5.4	5.1	5.1	5.5	5.7	5.9	5.8	5.5
120	5.1	5.1	5.7	5.6	6.3	6.7	5.7	5.4	5.7	5.9	5.8	5.9	6.0	5.8
Mean	4.7	4.7	5.6	5.5	6.0	6.0	-	5.0	5.1	5.6	5.6	5.7	5.8	-
L.S.D$_{0.05}$														
R				0.7							0.5			
N				0.5							0.4			
R×N				0.9							0.9			

C: control, WR: wheat residue, R: residue, N: nitrogen

Nitrogen uptake

The incorporation of wheat straw increased the N uptake of rice by 1–7 kg N ha^{-1} without legume residue, whereas with *Sesbania* green manure and mungbean residue it increased the N uptake of rice by 12–22 and 11–17 kg N ha^{-1}, respectively (Table 3). Thus, legume residues increased the efficiency of wheat straw.

Sesbania green manure alone increased the N uptake of rice over fallow by 9–13 kg ha^{-1}, whereas mungbean residue incorporation increased the N uptake of rice over summer fallow by 9–15 kg ha^{-1}. Sharma and Prasad (1999) also reported an increase in the N uptake of rice after *Sesbania* green manuring and mungbean residue incorporation. The nitrogen uptake by rice increased significantly when the rate of urea N was increased up to 60 kg N ha^{-1} in 1995–96 and to 120 kg N ha^{-1} in 1995–96. The interaction between crop residues and fertilizer N was not significant in respect of the N uptake of rice.

Wheat residue had no significant residual effect on the N uptake of succeeding wheat whether it was incorporated alone or with *Sesbania* green manure/mungbean residue. The incorporation of *Sesbania* green manure and mungbean residue significantly increased the N uptake of succeeding wheat by 11–12 and 13–15 kg N ha^{-1}, respectively.

The nitrogen uptake of succeeding wheat increased with an increase in the rate of N application to rice. The first increment of 60 kg N ha^{-1} increased the N uptake by 6–10 kg ha^{-1} whereas the second increment of 60 kg N ha^{-1} increased it by 3–6 kg N ha^{-1}. The interaction between crop residues and fertilizer N was not found to be significant in respect of the N uptake by succeeding wheat.

Soil organic C

Wheat residue increased the organic C content of the soil by 0.06–0.07%, *Sesbania* green manure (SGM) by 0.09–0.10%, SGM + wheat residue by 0.11–0.13%, mungbean residue (MBI) by 0.12–0.13% and MBI + wheat residue by 0.12–0.18%. Thus, soil organic carbon was enhanced by the incorporation of both wheat and legume residue, but the increase was the highest when wheat residue enriched with legume residue was incorporated. The application of N to rice at 120 kg N ha^{-1} also resulted in an increase in the organic C in the soil. Sharma et al. (2000) also reported such an increase probably due to higher root biomass with a higher rate of N application.

The interaction between crop residue and urea N was significant only in 1995–96 and showed that fertilizer N only had an effect on the organic C content of the soil when wheat residue enriched with *Sesbania*/mungbean residue was incorporated.

Table 3
Effect of fertilizer N on N uptake (kg N ha^{-1}) of rice and succeeding wheat as influenced by wheat, legume and legume-enriched wheat residue

N (kg ha^{-1})	1995							1996						
	Fallow		*Sesbania*		Mungbean		Mean	Fallow		*Sesbania*		Mungbean		Mean
	C	WR	C	WR	C	WR		C	WR	C	WR	C	WR	
							Rice							
0	81	76	94	97	90	90	73	65	74	84	95	89	89	83
60	92	90	99	102	102	107	98	78	87	93	102	94	93	91
120	95	97	104	105	103	105	102	93	96	95	105	98	106	99
Mean	89	90	99	101	98	101	–	79	86	92	101	94	96	–
L.S.D$_{0.05}$														
R				8							6			
N				5							5			
R×N				NS							NS			
							Wheat							
0	82	86	95	97	97	102	93	81	84	92	92	96	94	90
60	93	97	105	104	113	112	104	87	91	97	99	102	101	96
120	103	105	115	113	112	113	110	92	93	103	103	101	104	100
Mean	93	96	105	105	107	109	-	87	89	98	98	100	100	-
L.S.D$_{0.05}$														
R				5							6			
N				7							5			
R×N				NS							NS			

C: control, WR: wheat residue, R: residue, N: nitrogen

Soil Kjeldahl N

In 1994–95, there was no significant effect of crop residue and N application to rice on soil Kjeldahl N; however, the lowest Kjeldahl N was observed in plots in which wheat residue was incorporated and the highest in plots receiving wheat residue enriched with *Sesbania* green manure or mungbean residue. In 1995–96, *Sesbania* green manuring and mungbean residue incorporation significantly increased Kjeldahl N. The incorporation of wheat residue had no significant effect on the Kjeldahl N content of the soil. However, in both years the incorporation of mungbean + wheat residue resulted in the highest Kjeldahl N content in the soil. Nitrogen application to rice significantly increased the Kjeldahl N content of the soil. Glendining et al. (1996) also reported that the application of 144 kg N ha^{-1} to Broadbalk winter wheat for 22 years caused a 60% increase in mineralizable N. A significant interaction between the crop residue and fertilizer N applied to rice during 1995–96 indicated that the effect of fertilizer N on soil Kjeldahl N was significantly positive only after *Sesbania* green manuring and mungbean residue incorporation. Thus, the fertilizer N applied to rice was conserved better with the incorporation of legume residues.

Soil available P

Wheat residue incorporation and *Sesbania* green manuring had no significant effect on the available P content of the soil. However, when legume-enriched wheat residue was incorporated the available P content of the soil increased significantly over fallow (Table 4). Thus, the efficiency of wheat residue was increased by *Sesbania* green manuring. The incorporation of mungbean also increased the available P content of the soil. This could be due to greater CO_2 evolution from the decomposition of mungbean residue as compared with the decomposition of *Sesbania* green manure (IARI, 1996). The available P content of the soil also increased significantly when the rate of N application to rice was increased from 0 to 120 kg N ha^{-1}. This could be due to higher root mass with the higher rate of N, which could feed more on native soil P.

The interaction between the crop residue and fertilizer N applied to rice indicated that the effect of N application on the available P content of the soil was observed only when residues of both wheat and *Sesbania* were incorporated. In 1996–97, the effect of N application on the available P content was also significant when mungbean residue was incorporated.

Table 4

Effect of nitrogen on available P and available K in soil as influenced by wheat, legume and legume-enriched wheat residue

N	1995							1996						
(kg ha^{-1})	Fallow		*Sesbania*		Mungbean		Mean	Fallow		*Sesbania*		Mungbean		Mean
	C	WR	C	WR	C	WR		C	WR	C	WR	C	WR	
					Available P (kg ha^{-1})									
0	12	16	15	13	18	22	16	13	11	17	15	15	16	15
60	13	16	16	21	19	22	17	15	12	18	24	21	19	18
120	14	20	23	24	22	25	21	16	16	19	21	26	21	20
Mean	13	17	18	19	19	23	-	15	13	18	20	20	19	-
L.S.D$_{0.05}$														
R				6							5			
N				5							4			
R×N				9							8			
					Available K (kg ha^{-1})									
0	425	465	475	450	450	455	453	323	477	440	520	320	550	438
60	475	470	460	490	480	490	477	380	527	440	510	417	533	468
120	445	480	470	505	455	475	472	437	473	397	500	397	480	447
Mean	448	472	468	482	462	473	-	380	492	425	511	378	521	-
L.S.D$_{0.05}$														
R				NS							77			
N				NS							NS			
R×N				NS							NS			

C: control, WR: wheat residue, R: residue, N: nitrogen

Soil available K

About 90% of the total K uptake by wheat remains in the straw (Sharma and Prasad, 1980) and its incorporation increased the available K content of the soil. However, the increase in the available K content of the soil due to wheat residue incorporation was significant only in the second year of study (Table 4). Prasad et al. (1999) also reported an increase in soil available K due to wheat residue incorporation in the second year only of their study. This is understandable, because some time is required before measurable soil available K is built up. Nitrogen application to rice had no significant effect on the available K content of the soil.

The results thus show that the legume residue enrichment of wheat residue overcomes the adverse effect of the immobilization of native soil N by the incorporation of wheat residue with a higher C:N ratio; the benefits of this practice were observed in increased grain production and improved soil fertility.

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EFFECT OF ORGANIC MANURE AND SLOW-RELEASE N-FERTILIZERS ON THE PRODUCTIVITY OF WHEAT (*TRITICUM AESTIVUM* L.) IN SANDY SOIL

M. S. ZEIDAN and M. F. EL KRAMANY

FIELD CROPS RESEARCH DEPARTMENT, NATIONAL RESEARCH CENTER, DOKKI, GIZA, EGYPT

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Two field experiments were conducted during the two winter seasons of 1998/1999 and 1999/2000 in a private farm at El-Nagah Village, South El-Tahrir province, El-Behaira Governorate, Egypt. The aim of this study was to compare the effect of different forms of nitrogen fertilizer, i.e., ammonium sulphate 20.6% N, ammonium nitrate 33.5% N, and Enciabien 40% N (slow-release) with or without organic manure at 20 m^3/fed (4200 m^2) on the yield and nutrient contents of the wheat (*Triticum aestivum* L.) cultivar Sakha 69.

The results indicated that the use of organic manure surpassed the control which gave the highest number of spikes/plant, 1000-grain weight, grain yield (t/fed), crop index, harvest index, grain N, P and protein. The use of slow-release nitrogen fertilizer gave the highest 1000-grain weight, biological yield/plant, grain yield and biological yield (t/fed), grain N and protein if compared with other nitrogen sources. The highest tiller/plant, 1000-grain weight, grain yield (t/fed) and grain N and P gave the best results when slow-release nitrogen fertilizer was combined with organic manure at 20 m^3/fed.

Key words: organic manure, N fertilizer, wheat, sandy soil

Introduction

Wheat is one of the most important cereal crops in Egypt and fertilization is one of the important factors affecting its production.

Great efforts have been made by Egyptian scientists to improve wheat productivity by increasing the efficiency of added fertilizers by controlling the release or minimizing the loss of nutrients. The use of slow-release N fertilizers reduces N leaching from cropland, which contributes to the increase in the nitrate (NO_3) level in ground and surface water (Lichtenberg and Shapiro, 1997).

Depending on the concentration, these increases in N level can be unhealthy for humans. Nitrogen leaching from sandy soils is especially troublesome in the arid region. In order to decrease N leaching in these environments, farmers use split N application, utilize slow-release fertilizers and incorporate soil treatments such as organic manure. Recently, slow-release nitrogen fertilizer has been suggested as a potential N fertilizer for sandy soil that can control N leaching and increase N use efficiency (Perrin et al., 1997). On the other hand Ayyer (1992) reported that N losses from the soil could be controlled by coating soluble fertilizer with insoluble materials, thereby reducing its solubility and release into the soil. El-Karamity and Salem (1993) and Gately (1994) found that the application of ammonium sulphate increased grain yield better than ammonium nitrate or urea.

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Combining soluble and slow-release fertilizers is one way to provide wheat with the required nutrients throughout its growing season. The objective of this study was to determine the potential of slow-release N fertilizer compared with ammonium sulphate when combined with organic manure to meet the mid-season N requirements of wheat grown in sandy soils.

Materials and methods

Two field experiments were carried out at El-Nagah village, South El-Tahrir Province, El-Behaira Governorate, Egypt during two successive winter growing seasons of 1998/99 and 1999/2000 to study the response of wheat (*Triticum aestivum* L.) plants var. Sakha 69 to organic manure and different sources of nitrogen fertilizers in newly reclaimed sandy land. The soil of the experimental field was sandy loam in texture. The physico-chemical properties of the soil and the analyses of the composted organic garbage manure used are shown in Table 1. The treatments were arranged in a split plot design with four replicates with the organic manure treatments (20 m^3/fed=4200 m^2) in the main plots, while the combinations of nitrogen sources were allocated at random in the sub-plots. The plot size was 3×3.5 m. Each treatment received 30 kg P_2O_5 as single superphosphate and 50 kg K_2O/ha as potassium sulphate as a soil application before sowing.

Two N rates were applied (0–120 kg N/fed) and three sources were utilized, i.e. ammonium nitrate (NH_4NO_3, 33.5% N), ammonium sulphate [$(NH_4)_2SO_4$, 20.6% N] and Enciabien (40 % N), a slow-release N fertilizer obtained from the General Organization for Agriculture Equalization Fund (C.O.A.E.F), Egypt.

Random samples of ten guarded plants in each plot were taken at harvest to estimate the following characters :

1. Plant height (cm)
2. Number of tillers/plant
3. Number of spikes/plant
4. Number of spikelets/spike
5. Biological yield/plant
6. 1000-grain weight (g)
7. Grain yield (t/fed)
8. Biological yield (t/fed)
9. Crop index (%)
10. Harvest index (%)

Chemical analysis

The protein % in the grain was determined and calculated according to A.O.A.C. (1970). The P content was determined according to the method described by Frei et al. (1964). The data of the two experiments were subjected to statistical analysis according to Snedecor and Cochran (1967). The combined analysis was conducted for the data of the two experiments according to Cochran and Cox (1968). The least significant difference (LSD) was used to compare the means.

Table 1

Analyses of the experimental soil and organic manure fertilizer according to the methods given by Jackson (1971)

Properties	Organic manure	Soil
Fine sand (%)	–	94.8
Silt (%)	–	4.5
Clay (%)	–	0.7
Texture	–	Sandy
Organic matter (%)	17.5	0.48
pH	7.28	7.70
EC mmhos/cm^2	2.13	0.20
$CaCO_3$ (%)	2.30	3.20
Total N (ppm)	850	4.10
Available P (ppm)	110	4.30
K (mg/100 g soil)	405.1	6.5

Results and discussion

Effect of organic manure

Data on the growth, yield, yield components and chemical contents of the wheat plants as affected by organic manure are presented in Tables 2 and 3. Generally, the growth, yield, yield components and chemical contents of the wheat plants responded positively to the application of organic manure. Plants grown in soil treated with organic manure combined with the recommended NPK rate revealed the highest significant increase in plant height, number of tillers/plant, number of spikes/plant, number of spikelets/spike, 1000-grain weight, crop index (%), biological yield (t/fed), harvest index (%), grain yield (t/fed) and grain N, P and protein compared with control plants treated only with the recommended dose of NPK fertilizers. The stimulating influence of organic amendments on wheat grown in sandy soils might be attributed to the improved microbial activity in the soil which probably improves the availability of the nutrients (Brechelt, 1989; Hannaa-Mona, 1994). In addition Veleck et al. (1981) reported that the application of organic manure to the soils of the arid regions partially protected nitrogen from loss by the temporary fixation of mobilized nitrogen.

Tables 2 and 3 also indicate that using organic manure combined with the recommended dose of NPK fertilizers caused a significant increase in the N, P and protein contents in wheat grain as compared with the control plants (NPK only). Mengel and Kirkby (1979) reported that organic fertilizers such as farmyard manure contain potassium, magnesium and phosphate in an inorganic form. A similar trend was found by Abd El-Kariem (1989), El-Koumy (1998), Azad et al. (1998) and Dahdouh et al. (1999). The reduction in some traits of wheat as a result of treatment with the recommended NPK fertilizers without organic manure may be due to the leaching of the nutrients with the mass flow of water deep in the soil. Nutrients can be lost from the rooting zone, especially, on soils which drain rapidly, such as sandy soils.

Table 2

Effect of organic manure on yield components of Sakha 69 wheat
(Combined analysis of 1998/1999 and 1999/2000 seasons)

Treatments	Plant height (cm)	Number of			1000-grain weight (g)	Grain yield /plant (g)
		Tillers/plant	Spikes/plant	Spikelets/spike		
Organic manure	106.7	3.8	3.55	23.4	37.3	12.4
Control	103.1	3.5	3.36	23.3	35.7	11.8
$LSD_{5\%}$	NS	NS	0.11	NS	1.1	NS

NS = non-significant

Table 3

Yield, N, P and protein content in grains of wheat plants as affected by organic manure application (Combined analysis 1998/1999 and 1999/2000 seasons)

Treatment	GY (t/fed)	BY (t/fed)	CI (%)	HI (%)	Grain N conc. (g/kg)	Grain P conc. (g/kg)	Grain protein (%)
Organic manure	1.89	2.60	71.3	41.3	22.7	3.5	12.4
Control	1.72	2.42	70.4	38.3	19.4	2.9	9.3
$LSD_{5\%}$	0.09	NS	0.31	1.05	0.32	0.10	2.1

GY: Grain yield; BY: Biological yield; CI: Crop index; HI: Harvest index; NS = non-significant

Effect of nitrogen sources

The data in Tables 4 and 5 revealed significant differences due to N sources in all studied traits except plant height, number of spikelets/spike, crop index, harvest index and P concentration in the grains.

Wheat grown in soil amended with slow-release N fertilizer (Enciabein) gave significantly more dry weight/plant, 1000-grain weight, grain yield/fed, straw yield/fed, and N and protein content in the seeds than wheat grown in soil amended with ammonium nitrate or ammonium sulphate, while ammonium nitrate gave significantly more tillers/plant and spikes/plant compared with the control. On the other hand, a slight increase was only observed in a few traits due to ammonium sulphate fertilizer. Similar results were reported by Kolhe and Mittra (1989), who found that slow-release nitrogen fertilizer gave the highest yield of wheat. Ragasits and Berecz (1996) and Zhang et al. (1998) also showed that slow-release urea increased wheat yields by 18.3–27.8% and increased rice yields by 27.5–50.4% as compared with common urea. Perrin et al. (1997) showed that amending sandy soils with slow-release N can reduce N leaching, increase plant growth and increase N concentration compared with sweetcorn grown in soil amended with ammonium sulphate.

Table 4

Yield components of wheat plants as affected by N sources (Combined analysis of 1998/1999 and 1999/2000 seasons)

Parameters / N sources	Plant height (cm)	No. of Tillers /plant	No. of Spikes /plant	No. of Spikelets /spike	1000-grain weight (g)	Biological yield (g/plant)
Control	103.3	3.2	3.1	22.3	34.5	11.6
$(NH_4)_2SO_4$	106.8	3.6	3.6	23.5	36.5	12.0
NH_4NO_3	105.6	4.0	3.9	23.6	36.5	12.2
Slow-release N fertilizer	107.2	3.5	3.4	22.9	38.6	12.7
$LSD_{5\%}$	N.S	0.19	0.23	N.S	1.93	0.7

NS = non-significant

Table 5
Yield components, N, P and protein content in wheat grain as affected by N sources (Combined analysis of 1998/1999 and 1999/2000 seasons)

N source \ Parameters	Grain yield (t/fed)	Biological yield (t/fed)	Crop index (%)	Harvest index (%)	Grain N conc. (g/kg)	Grain P conc. (g/kg)	Grain protein (%)
Control	1.70	2.35	67.8	38.6	20.3	2.4	9.6
$(NH_4)_2SO_4$	1.80	2.50	70.1	40.5	21.7	2.6	10.0
NH_4NO_3	1.75	2.48	69.5	39.5	21.5	2.5	10.5
Slow-release N fertilizer	1.90	2.64	71.5	41.2	22.7	2.9	11.8
$LSD_{5\%}$	0.10	0.12	N.S	N.S	0.33	N.S	0.10

NS = non-significant

Interaction effect

The data listed in Table 6 show significant interactions between organic manure and sources of nitrogen in the number of tillers/plant, 1000-grain weight, grain yield (t/fed), grain N and P concentration.

The results in Table 6 clearly indicate that the application of organic manure and slow-release nitrogen fertilizer (Enciabien) was the best treatment, producing the highest number of both tillers and spikes per plant, 1000-grain weight, grain yield/fed and the maximum contents of nitrogen and phosphorus in the grain.

Table 6
Yield components and N, P concentration in wheat grains as affected by organic manure and sources of nitrogen (Combined analysis of 1998/99 and 1999/200 seasons)

Treatment	Number of		1000-grain wt.(g)	Grain yield (t/fed)	Grain N (conc.g/kg)	Grain P (conc.g /kg)
	Tillers/plant	Spikes/plant				
Without organic manure						
Control	3.0	2.9	33.5	1.61	17.1	3.3
$(NH_4)_2SO_4$	3.5	3.2	36.5	1.73	19.1	4.2
NH_4NO_3	3.8	3.1	35.9	1.68	19.1	4.2
Slow-release N fertilizer	3.4	3.3	38.6	1.82	19.3	4.3
With organic manure						
Control	3.3	3.1	35.6	1.73	19.4	4.2
$(NH_4)_2SO_4$	3.9	3.5	37.3	1.82	21.8	6.5
NH_4NO_3	3.7	3.4	37.5	1.84	21.8	6.5
Slow-release N fertilizer	4.3	3.0	40.3	1.95	22.8	6.7
$LSD_{5\%}$	0.2	0.3	1.8	0.10	0.3	0.32

Conclusions

Recently the use of organic manure in agriculture has received considerable attention because of the environmental problems associated with alternative disposal methods and the harmful effect on human health. On the other hand, organic manure improves the physical condition of the soil, thus increasing the water-holding capacity, preventing nutrient leaching and adding more mineral nutrients to the soil. In addition, amending sandy soil with slow-release nitrogen reduces N leaching and increases the yield of wheat in such a poor soil. Also the effect of slow-release N is positive from both environmental and economical aspects. At present in wheat production the split application of traditional N fertilizers is cheaper, but slow-release N fertilizers, when applied in optimal doses at the right time, minimize the risk of N losses by leaching. Slow-release N fertilizer has long-term effects including reduced leaching losses and enhanced N uptake, as well as positive effects on both health and soil nutrient levels. Therefore combining slow-release N fertilizer with organic manure could be effective in eliminating mid-season N deficiency.

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Short communication

WHEAT RESPONSE TO 2,4-D APPLICATION AT TWO GROWTH STAGES UNDER SEMI-ARID CONDITIONS

M. A. TURK and A. M. TAWAHA

DEPARTMENT OF PLANT PRODUCTION, FACULTY OF AGRICULTURE, JORDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, IRBID, JORDAN

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A 2-year field study was conducted during the rainy seasons of 1999 and 2000 at Houfa in northern Jordan, to study the performance of two wheat cultivars ACSAD 65 and F8 and their response to hand weeding (practised monthly during the growing seasons) and 2,4-D application at different growth stages. In both growing seasons, no significant differences ($P \leq 0.05$) in grain yield were recorded between the cultivars studied. Differences in weed number and fresh weight were significant between the various treatments in both seasons. Hand weeding proved the best method of weed control. In both growing seasons, yield reductions occurred when 2,4-D was applied to wheat, irrespective of the stage of application. Hand weeding treatment was more effective than 2,4-D application in suppressing weed growth.

Key words: *Triticum,* grain, weed, 2,4-D

Introduction

Wheat is grown under rainfed conditions in the north of Jordan, where limited precipitation restricts yield. Weeds may reduce yields further by competing with wheat for available moisture. Therefore, effective weed control is important in optimizing rainfed wheat yields. Phenoxy herbicides have been used for broadleaf weed control in wheat since the late 1940s; however, their misapplication can reduce yields (Klingman and Ashton, 1982). Wheat is susceptible to phenoxy herbicide injury from emergence to the four-leaf stage and from jointing to the soft dough stage of growth (Martin et al., 1990). Phenoxy herbicide application at these stages may reduce plant height, delay maturity and reduce grain yield (Klingman and Ashton, 1982). Plants treated with 2,4-D often exhibit malformed leaves, stems and roots. 2,4-D affects the plant metabolism by stimulating nucleic and protein syntheses, which affects the activity of enzymes, respiration and cell division (EPA, 1988). Cells in the phloem of treated plants are often crushed or plugged, interfering with normal food transport (Mullison, 1987), which can leave parts of the plant malnourished or possibly lead to death. The crop safety of 2,4-D on the newer cultivars grown in Jordan has not been evaluated and growers have reported injury to wheat crops when the recommended herbicide has been used. Therefore, the objective of this research was to study the performance of two wheat cultivars and their response to hand weeding and 2,4-D application at different growth stages.

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Materials and methods

Field experiments were conducted at Houfa in northern Jordan during the two growing seasons of 1998/1999 and 1999/2000. The location has a Mediterranean climate of mild rainy winters and dry hot summers. The experimental field received granular fertilizer (DAP: diammonium phosphate, 18% N and 46 % P_2O_5) at a rate of 100 kg ha^{-1}, which was mixed with the soil prior to planting. Split plot designs with three replications were used in both years. The cultivars, ACSAD 65 and F-8, were randomly assigned to the main plots in each replicate. Herbicide and hand weeding treatments were randomly assigned to each cultivar plot, representing the subplot treatments. Each subplot consisted of four rows, 30 cm apart and 2 m in length. The seed were sown by hand at a seeding rate of 100 kg ha^{-1} on 25 Nov. 1999 and 27 Nov. 2000. The alleys between the replicates were 1 m wide. The dominant broadleaf weed species were *Cardaria draba* L., *Diplotaxis erucoides* L., *Moluccella leavis* L. and *Brassica nigra* L. The dominant grass weed was *Hordeum murinum* L. The weed treatments were: weed check (untreated), hand weeding (practised monthly during the growing season) and 2,4-D (2,4-dichlorophenoxy acetic acid) applied at a rate of 480 g ha^{-1} (a. i.) to the wheat cultivars at two growth stages. The herbicides were applied with a mounted sprayer equipped with a fan-type nozzle. The plots were evaluated visually on a 0 to 100 scale (where 0 = no injury and 100 = plant death) to estimate wheat injury 21 days after the treatment. The wheat growth stages were identified using the scale described by Zadoks et al. (1974). The wheat growth stages and herbicide application dates were stage 13 (3-leaf) on Jan. 10, 1999 and Jan. 11, 2000, and stage 29 (tillering) on Feb. 10, 1999 and Feb. 11, 2000.

The measurements recorded each year included grain yield (kg ha^{-1}), spikes m^{-2}, grains spike^{-1}, plant height (cm), spike length (cm), and days to 50% heading. Before harvest, the weed number and fresh aboveground biomass were determined in four 0.25 m^{-2} random quadrates per plot. The weed control efficiency (WCE) was calculated using the following formula, as reported by Singh et al. (2000):

$$\frac{\text{Dry matter of weeds in unweeded plot} - \text{Dry matter of weeds in treatment}}{\text{Dry matter of weeds in unweeded plot}} \times 100$$

The plants were harvested at maturity on July 13 and July 15 in the 1999 and 2000 growing seasons, respectively. The plant of one m^2 quadrate from the three central rows of each plot were clipped 10 cm above the soil surface with a hand sickle. The MSTAT-C program was used for statistical analysis. The data for each trait were analysed for a randomized complete block design (RCBD) with a split plot arrangement according to the procedure outlined by Steel and Torrie (1980). Comparisons between means were made using least significant differences (LSD) at the 0.05 probability level.

Results and discussion

The 1999–2000 growing season was cooler and wetter than 1998–1999, with seasonal precipitation totals of 184.8 mm and 342.6 mm, in the first and second growing season, respectively. The annual variation of rainfall in Jordan is very high, especially in areas with low rainfall (Turk, 1998). The high interannual variability of rainfall and its erratic distribution are major reasons for low wheat yields. More than 78.2% of the seasonal precipitation in the 1999–2000 season was concentrated in January and February, while in the 1998–1999 season only 60.3% fell in these months (data not shown). In both growing seasons, plants in stage 13 (3-leaf) and stage 29 (tillering) were susceptible to 2,4-D injury. Treatment at the 3-leaf stage caused more visible injury than treatment at the tillering stage (data not shown). The results are in accordance with those of Martin et al. (1990) and Klingman and Ashton (1982).

No significant differences ($P \leq 0.05$) in grain yield were recorded between the cultivars studied in either growing season. In both growing seasons, the grain yield was affected by the weed control methods. Hand weeding provided the best weed control and this was reflected in higher wheat yields in both seasons. The increase in grain yield after hand weeding was mainly due to the effective control of weeds with reductions in both weed dry matter and weed intensity, resulting in more spikes m^{-2} and grains $spike^{-1}$ (Table 1) and thus higher grain yield. The effect of weed removal on the wheat yield was more obvious in the second growing season, where the wheat yield in weedy plots was 22.0 % less than after hand weeding, compared to an 8.5% reduction in the first growing season. The reduction in wheat yield due to weed interference was greater in the second season, which was characterized by more favourable weather conditions, allowing the weeds to grow and suppress the weak, slow-growing wheat plants. Significant differences in spikes m^{-2} and grains $spike^{-1}$ due to weed interference were observed in both growing seasons (Table 1). Weedy plots had less spikes m^{-2} and grains $spike^{-1}$ compared to hand-weeded plots. In both growing seasons, 2,4-D treatment applied at stage 29 (tillering) decreased the wheat yield by 9.3 and 1.3%, respectively. The lowest grain yield was recorded when 2,4-D was applied at growth stage 13 (3-leaf), with yield reductions of 13% and 3.6%, respectively. Wheat yield losses due to 2,4-D were closely related to reductions in the number of grains $spike^{-1}$ and spikes m^{-2} (Table 1).

No significant differences ($P \leq 0.05$) in plant height and spike length were recorded between the cultivars studied in either growing season (Table 2). Plant height and spike length were markedly increased in the weed-free plots compared with the other treatments (Table 2).

Table 1

Yield and yield components for two wheat cultivars as affected by hand weeding and 2,4-D application at two growth stages

		Grain yield (kg ha^{-1})		Grains $spike^{-1}$		Spikes m^{-2}	
		1999	2000	1999	2000	1999	2000
Cultivars	F8	1018.8a	1140.0a	34.0a	36.0a	443.8b	483.8b
	ACSAD 65	1056.3a	1162.5a	29.3b	31.3b	487.5a	512.5aa
	LSD (0.05)	NS	NS	3.7	3.1	23.0	23.3
Treatments	Control	1075b	1090b	34.5b	36.5b	475.0b	485b
	Hand-weeding	1175a	1390a	38.0a	40.0a	525.0a	580a
	2,4-D ester at stage 13	925c	1050c	25.0d	27d	437.5b	452.5bc
	2,4-D ester at stage 29	975c	1075c	29.0c	31.0c	425bc	475b
	LSD (0.05)	89	40	3.2	3.4	44	48
	Cultivar × Treatment	NS	NS	NS	NS	NS	NS

Means in each column followed by the same letters were not significantly different according to LSD ($P \leq 0.05$). NS = non-significant

Table 2
Phenological traits for two wheat cultivars as affected by hand weeding and 2,4-D application at two growth stages

		Plant height (cm)		Spike length (cm)		Days to 50 % heading	
		1999	2000	1999	2000	1999	2000
Cultivars	F8	79.0a	81.5a	7.3a	7.3a	106.8a	109.0a
	ACSAD 65	79.3a	81.3a	6.5a	7.0a	105.0a	108.0a
	LSD (0.05)	NS	NS	NS	NS	NS	NS
Treatments	Control	80.5b	82.5b	7.5b	8.5a	103.0b	107.0b
	Hand-weeding	84.5a	86.5a	9.0a	9.0a	103.0b	104.0c
	2,4-D ester at stage 13	75.0c	78.0d	4.5c	4.5c	109.0a	112.0a
	2,4-D ester at stage 29	76.5c	80.0c	6.5b	6.5b	110.0a	112.0a
	LSD (0.05)	3.5	2.0	1.4	1.2	2.5	2.9
	Cutivar × Treatment	NS	NS	NS	NS	NS	NS

Means in each column followed by the same letters were not significantly different according to LSD ($P \leq 0.05$). NS = non-significant

On the other hand, 2,4-D application reduced plant height and spike length. The significantly lowest plant heights were recorded after the application of 2,4-D at growth stage 13 (3-leaf) in both years (Table 2). The results of this study were in agreement with those obtained by Klingman and Ashton (1982), who found that 2,4-D application can reduce plant height. This may be due to the fact that 2,4-D and other auxin-type compounds inhibit cell division and growth, usually in the meristematic regions. No significant differences in days to 50% heading were recorded between the cultivars studied in either growing season. The heading of both cultivars was delayed when 2,4-D was sprayed in both growing seasons, irrespective of the time of application.

Differences in weed number and fresh weight were significant between the various treatments in both seasons (Table 3). Hand weeding proved significantly superior to the control treatment, reducing the weed number and fresh weight of weeds compared to the control. In addition, the hand-weeded treatment was more effective than 2,4-D applications in suppressing weed growth. On the average of two years, the weed control efficiency ranged from 53.6 to 90.7. The maximum weed control efficiency of 90.7 was recorded for hand weeding, whereas it was 85.8 in the case of 2,4-D application at stage 13 (3-leaf). Minimum weed control efficiency was recorded for the application of 2,4-D applied at growth stage 29 (tillering) in both growing seasons.

In conclusion, using suitable high yielding cultivars which can withstand moisture stress or erratic rainfall during the growing period is a major means of improving wheat yields. Hand weeding, which is practised by traditional farmers, proved to be superior when compared to 2,4-D, irrespective of the stage of application, under the conditions that prevailed in this study. In both growing seasons 2,4-D application at stage 13 (3-leaf) and stage 29 (tillering) was associated with yield reductions due to a reduction in spikes m^{-2} and grains $spike^{-1}$. These results encourage farmers to practise hand weeding, which gave the best yields in both seasons.

Table 3
Number and weight of weeds (g m^{-2}) for two wheat cultivars as affected by hand weeding and 2,4-D application at two growth stages

		Number of weeds		Weight of weeds		WCE (%)*		WCE (%)*
		1999	2000	1999	2000	1999	2000	Mean
Cultivars	F8	26.8a	37.4a	626.4a	860.2a	–	–	–
	ACSAD 65	26.3a	39.4a	614.9a	906.2a	–	–	–
	LSD (0.05)	NS	NS	NS	NS	–	–	–
Treatments	Control	52.5a	73.0a	1207.5a	1679.0a	–	–	–
	Hand weeding	13.5c	20.0c	110.5c	160.0c	90.8	90.5	90.7
	2,4-D ester at stage 13	16.0c	29.0b	568.0b	667.0b	60.3	60.3	56.7
	2,4-D ester at stage 29	26.0b	31.5b	599.0b	724.5b	56.3	56.8	53.6
	LSD (0.05)	3.7	3.6	133	207	–	–	–
	Cultivar × Treatment	NS	NS	NS	NS	–	–	–

Means in each column followed by the same letters were not significantly different according to LSD ($P \leq 0.05$). *Data not analysed statistically. NS = non-significant; WCE: weed control efficiency

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Short communication

ENHANCEMENT OF GROWTH OF ONION (*ALLIUM CEPA* L.) BY BIOLOGICAL CONTROL AGENT *TRICHODERMA* SPP.

E. PAYGHAMI, S. MASSIHA, B. AHARY, M. VALIZADEH and A. MOTALLEBI

DEPARTMENT OF PLANT PROTECTION, FACULTY OF AGRICULTURE, TABRIZ UNIVERSITY, TABRIZ, IRAN

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The effect of *Trichoderma harzianum* and *Trichoderma viride* (isolated from mycoflora in the rhizosphere of onion) in increasing the growth of onion was studied in a completely randomized design in pots with 12 replications under greenhouse conditions at 21°C with a 12-h light/dark cycle (fluorescent and incandescent lighting). The biological control of *Sclerotium cepivorum* Berk, the causal agent of white rot of onion, was also investigated in this experiment. The addition of *Trichoderma* spp. to autoclaved soil (inoculation of 2/3 of the top soil in the pots with 4% (v/v) inoculum of *T. harzianum* and *T. viride*) significantly increased the growth and fresh weight of the onion plants (P=1%). The biological control of *S. cepivorum* was achieved with *T. harzianum* and *T. viride,* but no significant difference was observed between the two species.

Key words: *Sclerotium cepivorum, Trichoderma harzianum, Trichoderma viride,* white rot of onion

Introduction

Trichoderma spp. are biological control agents effective against *Fusarium* spp. (Sivan et al., 1987), *Rhizoctonia solani* (Henis et al., 1978) and *Pythium ultimum* (Bell et al., 1982). It has been indicated that they increase seed germination and enhance plant growth independent of any plant disease (Baker, 1988; Windham et al., 1985)

In recent years damage by *Sclerotium cepivorum* Berk, the causal agent of white rot in onion, has increased in the Elkhichy region (Tabriz province of Iran).

The object of this research was to investigate the effect of *Trichoderma* spp. in increasing the growth of onion. The possibility of the biological control of *S. cepivorum* by *Trichoderma* spp. was also investigated.

Materials and methods

Sandy loam soil was used in all the experiments. The soil was air-dried, sieved through a 4 mm mesh screen, moistened to approximately 15% water, sterilized at 85°C for 30 minutes and exposed to the air for 1 week before use in the pathogenicity test.

Samples infected with *S. cepivorum* were collected and the causal agent was isolated. The fungus was propagated on sterilized wheat seeds and mixed with sterilized soil (wheat seed 100 g, soil 900 g, water 160 ml) for the pathogenicity test and to produce inoculum. The antagonistic effects of *Trichoderma* spp. *(T. viride* and *T. harzianum)* were investigated using the double culture method (Bell et al., 1982). The inocula of *T. viride* and *T. harzianum* used to control *S. cepivorum* were propagated on wheat bran and soil (wheat bran 100 g, water 160 ml and soil 900 g). The species were isolated from the mycoflora in the rhizosphere of onion using the method of Davet (1979). All the growth-room experiments were conducted in 17.5 cm square plastic pots with 12 replications per treatment. One onion (*Allium cepa* L.) seed disinfected with 0.5% sodium hypochlorate solution was planted in each pot after the soil was moistened. The plants were grown under greenhouse conditions at 21°C with a 12-h light-dark cycle (fluorescent and incandescent lighting).

The effect of *Trichoderma* spp. on the biocontrol of *S. cepivorum* and on the enhancement of onion growth was studied in a completely randomized design under greenhouse conditions. Two-thirds of the top soil in the pots was inoculated with 4% (v/v) inoculum of the pathogen and *Trichoderma* spp.

Results

Isolates belonging to two species: *Trichoderma viride* and *T. harzianum* were selected from those isolated on the selective medium from onion bulbs and the soil surrounding them. These isolates were found on samples taken in the Elkhichy region. Each isolate was tested *in vitro* from the standpoint of parasitic activity (hyperparasitism) and inhibitory effect of extracellular metabolites (antibiosis) on *S. cepivorum*. The results exhibit three forms of antagonism towards *S. cepivorum,* the causal agent of white rot on onion: competition, coiling (deformation) and lysis.

Under greenhouse conditions, the biocontrol of *S. cepivorum* by *T. harzianum* and *T. viride* 20 weeks after planting showed that the antagonistic fungi decreased white rot disease by 50 and 37.5%, respectively, compared to the control (Fig. 1), but no significant difference was observed between *T. harzianum* and *T. viride*. Increased growth of onion was obtained when the pot soil was treated with an inoculum of *T. harzianum* and *T. viride,* and the plants resulting from treated soils produced significantly more fresh weight (Fig. 2).

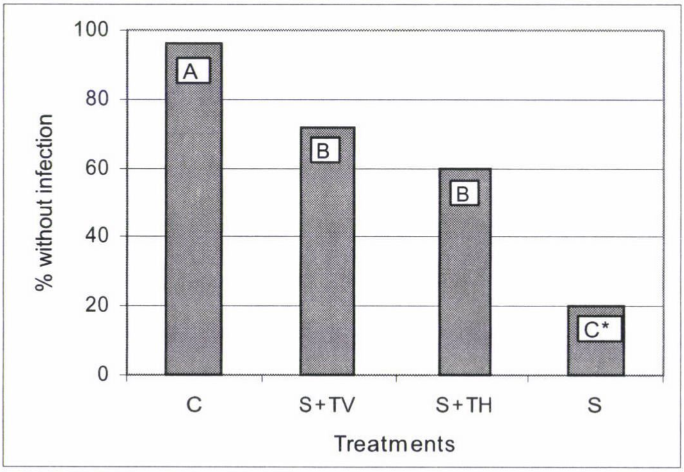

Fig. 1. Comparison of the average percentage without infection (C = control: no inoculation) by *S. cepivorum* (S) as influenced by *Trichoderma viride* (TV) and *T. harzianum* (TH); * = Treatments with different letters were significantly different according to Duncan's multiple range test (P<0.01)

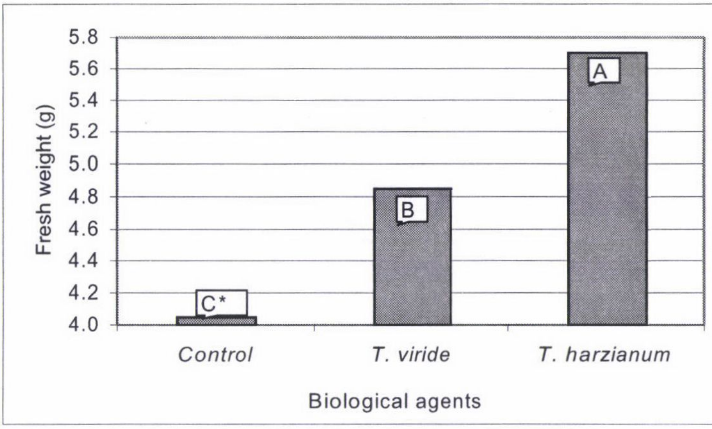

Fig. 2. Average fresh weight of onion plants after treating soils with *T. viride* and *T. harzianum;* * = Treatments with different letters were significantly different according to Duncan's multiple range test ($P<0.01$).

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Review

EFFECTS OF PLANT DENSITY, ROW SPACING AND ROW ORIENTATION ON YIELD AND ACHENE QUALITY IN RAINFED SUNFLOWER

M. LONG[1], B. FEIL[1] and W. DIEPENBROCK[2]

[1] INSTITUTE OF PLANT SCIENCES, ETH ZURICH, ZURICH, SWITZERLAND
[2] INSTITUT FÜR ACKER- UND PFLANZENBAU, MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, HALLE (SAALE), GERMANY

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A large body of literature on the responses of yield and other agronomic traits to planting patterns in rainfed sunflower (*Helianthus annuus* L.) is reviewed. Extensive studies on the effect of plant density on yield have given inconsistent results which are still not well explained by other agronomic traits. The effects of row spacing on yield and other traits seems to depend greatly on the environment where the trial is conducted and on the cultivar used. Information on the effect of row orientation is scarce and needs to be further studied over a large range of latitudes with the assistance of simulating models.

Key words: sunflower (*Helianthus annuus* L.), plant density, row spacing, row orientation

Management decisions such as the choice of plant density, row spacing and row orientation can affect the canopy structure (Wells et al., 1993), which may change the growth and yield of sunflower (*Helianthus annuus* L.). Many studies have focused on determining the effects of planting patterns on yield, but no consistent conclusions have been drawn (Long, 1999). Other studies are still in progress, so it is important to re-consider the overall effects of planting patterns on yield and other agronomic traits, particularly for rainfed sunflower where the environments are greatly variable.

1. Plant density

Plant density is a major management variable used in matching crop requirements to the environmental resources available. Plant density has been studied continuously and improved practically over the last century. For decades, research studies have demonstrated inconsistent responses of yield and other agronomic traits to plant density (Table 1). In the following, the characteristics of yield and other traits due to various plant densities will be briefly outlined.

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Table 1

Results of field studies on yield-plant density relationships in sunflower in the last 30 years

Plant density	Results / Conclusions	Sources
1.8, 2.5, 3.2, 5.6, 9.8, 12.5	Optimum AY at 6.0 to 7.5 plants m^{-2}	Vijayalakshmi et al. (1975)
2.5, 5.0, 7.5,10.0	AY and OY decreased with increasing PD	Alessi et al. (1977)
2.5, 5.0, 7.5, 15.0	Highest AYand OY with 2.5 and 5.0 plants m^{-2}	Jessop (1977)
2.6, 4.8, 7.2	Oil concentration unaffected by PD; TAW decreased with increasing PD	Miller and Fick (1978)
	Optimum AY at 4.0 to 6.0 plants m^{-2}	Radfore (1978)
1.7, 2.3, 3.7, 4.9, 6.2	AY increased with increasing PD	Robinson et al. (1980)
4.0 to 8.5	No significant effect of PD on AY; oil concentration increased with increasing PD, while TAW decreased with increasing PD	Holt and Campbell (1984)
2.9 to 7.3	AY and OY constant, TAW decreased with increasing PD	Miller et al. (1984)
3.75, 7.50	AY increased, while TAW decreased with increasing PD	Holt and Zentner (1985)
5.5, 11.1	No effect of PD on oil concentration; TAW decreased with increasing PD	Narwal and Malik (1985)
1.0, 3.5, 5.0	Maximum AY with 1.0 and 3.5 plants m^{-2}	Spackman (1985)
3.0, 4.5, 6.0, 7.5	No effect of PD on AY; oil concentration increased with increasing PD, while TAW decreased	Gubbels and Dedio (1986)
1, 3, 5, 7, 9, 11	Site-specific responses of AY at 9 locations to PD	Wade and Foreman (1988)
3.2, 4.9, 6.7, 8.4, 10.1	Semidwarf and conventional height cultivars responded similarly to increasing PD with decreasing AY per plant	Majid and Schneiter (1988)
3.0, 4.5, 6.0	TAW decreased sharply from 3.0 to 4.5 plants m^{-2}, but less pronouncedly from 4.5 to 6.0 plants m^{-2}; oil concentration increased more between 3.0 and 4.5 plants m^{-2} than between 4.5 and 6.0 plants m^{-2}	Gubbels and Dedio (1989)
5.5, 7.4, 14.8	Oil concentration increased, TAW decreased with increasing PD; AY increased from 5.5 to 7.4 plants m^{-2}, and remained constant at 14.8 plants m^{-2}	Gubbels and Dedio (1990)
4, 8, 12, 16	Highest AY with 8 plants m^{-2}	Tenebe et al. (1996)
3.12, 4.16, 6.25	AY increased, TAW decreased with increasing PD	Ortegón and Diaz (1997)
2.8, 3.4, 5.6, 11.1	Highest AY at 11.1 and 5.6 plants m^{-2}	Reddy et al. (1997)
4, 8, 12	Highest AY usually at 4 or 8 plants m^{-2}	Long (1999)

Abbreviations: AY=achene yield, OY=oil yield, PD=plant density, TAW=1000-achene weight

1.1. Responses of yield and yield components

Inconsistent responses of yields to increasing plant density probably indicate that the optimum plant density is dependent on water, light, nutrients, space, mutual shading and cultivar (Tenebe et al., 1996). Wade and Foreman (1988) observed that with increasing plant density the yield rose to a maximum, where it remained constant under favourable environmental conditions. Under less favourable conditions, however, the yield started to decline at very high plant density. Due to interplant competition for light, water and other yield-determining factors, the yields of individual plants will generally decrease with increasing plant density. However, lower yields per plant do not necessarily mean lower yields per area. Achene yield is a function of the number of capitula, the number of achene per capitulum (combined together as the achene number) and the weight of each achene (1000-achene weight). Maximum achene yield will be achieved when the combination of achene number and 1000-achene weight, which interact closely, is optimized. Thereafter, achene number, 1000-achene weight and oil concentration in the achene are the principal components of oil yield in sunflower. These components are determined sequentially during the life cycle of the crop (Diepenbrock and Pasda, 1995).

Manipulation via changing plant density is a major experimental approach to study the characteristics of all yield components. The sunflower plant has a tremendous ability to modify yield components in response to a wide range of environmental conditions. As a result, many studies have indicated no statistically significant differences in yields in response to varying plant density (Holt and Zentner, 1985; Miller and Fick, 1978; Rao and Saran, 1991; Robinson et al., 1980; Zaffaroni and Schneiter, 1991). For instance, sunflower is able to adjust to a low plant density by increasing both achene number and 1000-achene weight (Robinson, 1978). The linear decrease in 1000-achene weight with increasing plant density generally corresponded to an increase in oil concentration due to a "dilution" effect (Gubbels and Dedio, 1986; Diepenbrock et al., 2001). From the physiological point of view, sunflower crops compensate for low plant densities by increasing the size of the capitulum (Miller et al., 1984; Diepenbrock, 1987, 1988; Diepenbrock et al., 2001) and thus, probably achene number and 1000-achene weight. The number of achenes per capitulum decreased with increasing plant density due to a decrease in the number of flowers and in the fertility rate in the central part of the head (Villalobos et al., 1994).

1.2. Other agronomic traits

Apart from the interaction of yield components, phenotypic plasticity, expressed in adjustments in other agronomic traits, may also be involved in the inconsistent responses of yields to various plant densities. Phenotypic plasticity is a general characteristic of species in the genus *Helianthus* (Heiser et al., 1969) that is also present in commercial sunflower (Connor and Sadras, 1992).

First of all, the height of the plants can be greatly modified by changing plant density, while this interacts with the environment, in particular with soil water conditions. For instance, the plant height was significantly higher at high plant density than at low plant density before flowering due to higher plant competition within the row and to non-limited soil water (Vijayalakshmi et al., 1975). This trend was maintained at a later stage if soil water was not limited (Long, 1999) but reversed if soil water became seriously deficient (Vijayalakshmi et al., 1975).

Second, the diameter of the stems may respond to a change in plant density. For instance, stem diameter at low plant density was larger than at high plant density (Vijayalakshmi et al., 1975; Long, 1999). This trend was evident throughout the growth period and was possibly due to better soil water availability at low plant density (Vijayalakshmi et al., 1975; Long, 1999). Periodic soil moisture sampling showed that soil water was depleted most rapidly at high plant density (Vijayalakshmi et al., 1975; Long, 1999). In addition, plant density may change root distribution due to the availability of soil water. About 40% of the roots were in the 0–13 cm vertical layer at high plant density, while only 20% were in this layer at low plant density (Vijayalakshmi et al., 1975). This may help to explain why wilting is generally more prominent among plants at high density (Long, 1999).

1.3. Interaction with the yielding environment

Various yielding environments may play a role in the response of the yield to plant density. Sunflower crops can be expected to respond favourably to high plant densities when they are grown in environments providing optimum soil water, temperature and nutrients, and free of weed, disease and insect pests. Consequently, the minimum plant density required for maximum yield may vary across environments. For instance, some minimum plant densities for high yields were recommended as follows: 6.2 plants m^{-2} at Waseca and Grand Rapids, 3.7 at Lamberton, 4.9 at Morris and Crookston and 2.5 at Beckerl (Robinson et al., 1980). It should be borne in mind that plant densities above those required for maximum yield did not result in a reduction in grain yield, though they did reduce the achene number and 1000-achene weight. Therefore, the risk of yield reduction may be much less from seeding at the optimum or above optimum plant densities than seeding at too low plant densities.

2. Row spacing

Narrowing the row spacing may increase yield if light utilization is the only yield-limiting factor. Various experiments indicate that reducing the row spacing may result in a higher interception of light (reviewed by Long et al., 2001). However, the yield responses to various row spacings are inconsistent (Table 2), possibly due to changes in locations and cultivars.

Table 2
Results of field studies on yield-row spacing (cm) relationships in sunflower in the last 30 years

Row spacing	Results / Conclusions	Sources
35 to 60	Recommended RS for optimum AY	Vijayalakshmi et al. (1975)
30, 90	AY and OY decreased with increasing RS	Alessi et al. (1977)
36, 108	Higher AY with 36 cm RS	Radfore (1978)
50, 100	No effect of RS on AY	Robinson et al. (1982)
45, 90	No influence of RS on AY for late hybrid, but higher AY for early hybrid with wide RS	Gubbels and Dedio (1988)
30, 60	No RS effect on AY and oil concentration	Gubbels and Dedio (1990)
50, 75, 100	Highest AY usually at 75 or 100 cm RS	Long (1999)

Abbreviations: AY=achene yield, OY=oil yield, RS=row spacing

2.1. Yield traits

Theoretically, when using an ideal plant density under most production systems, maximum yield should occur when the spacing of the plants between rows and within the row is equal, i. e. equidistant spacing. Such equidistant arrangement can reduce intraplant competition for space, light and soil water during early growth stages. Furthermore, an equidistant spacing can increase light interception, reduce water losses due to evaporation and runoff, and reduce soil erosion. Owing to the many factors involved, the equidistant arrangement for a desired plant density may not always result in the highest yield. For instance, plants from rows spaced 36 cm apart outyielded those from rows spaced at 108 cm (Radfore, 1978). However, there was no difference in yields between 30 and 60 cm row spacings at various plant densities (Gubbels and Dedio, 1990). In contrast, non-equidistant arrangements seemed to produce higher yields than equidistant ones (Long, 1999).

2.2. Other agronomic traits

Robinson et al. (1982) suggested that plant height might be involved in the inconsistent responses of yields to row spacings, while there may exist an interaction between row spacing and plant density (Long, 1999). Over all plant densities, plant height increased with increasing row spacing (Long, 1999). Apart from the changes in plant height, root development and the response of plants to moisture stress during early growth and at flowering may also be modified by various row spacings. During the early seedling stage, when moisture is not limited, plants in equidistant planting may grow more vigorously and exhaust most of the available soil water. In a wide row spacing, i.e. non-equidistant arrangement, the competition for soil water within the row may become increasingly severe and thus the early growth may be restricted. However, in such cases the roots may be forced to go deep, and consequently plants in the wide row spacing may be better able to withstand prolonged dry periods later in the growing season. For instance, at anthesis the roots extended to a distance of 25 cm between the rows and to a depth of 50 cm in an equidistant arrangement of 36.7 × 36.7 cm. Meanwhile, in a non-equidistant arrangement of 135 × 10 cm, roots extended up to 70 cm between rows and to a depth of 60 cm (Vijayalakshmi et al., 1975). Interestingly, the authors estimated that 70% of the roots by weight were contained in a volume bounded by a lateral radius of 15 cm and a depth of 10 cm in the narrow rows, and by a 30 cm spread between rows and a 25 cm depth in the wide rows.

3. Row orientation

The row orientation of the sunflower crop may have an impact on light interception and soil water availability, especially during pre-anthesis growth, because active leaf movement results in light competition within or between rows as a function of the direction of light. Consequently, the yield and other traits are expected to respond to various row orientations. Under rainfed conditions, soil water may be the most limiting factor. Sunflower crops do not use water sparingly, because, under high evaporative demand, they maintain open stomata throughout the day, which results in an inefficient use of water and possibly increased water stress in the late developmental stages (Rawson and Constable, 1980). In addition, evaporation is affected either by soil water availability or by soil temperature, which is highly dependent on the radiation penetrating into the soil surface (Khalifa, 1984; Long and Eiszner, 2001). As a result, the light flow is the key issue which determines the yield and other traits. In the following, only the light conditions in two row orientations, east-west and north-south, will be elucidated from theoretical analysis and field experiments.

3.1. Theoretical analysis

Row orientation modifies the extent to which the canopy shades the ground. Its effect is related to solar height, which varies with time of day, date, latitude and the composition of the light (direct or diffuse). The effect of row orientation on light interception changes with crop development. When the sunflower plants are still small, the effect of row orientation is zero or very weak, due to wide interplant spacing and leaf phototropic movement. Later in the season, the leaves of neighbouring plants within the row start to overlap. During this period, row orientations may affect light interception. After the canopy is fully developed, the effect of row orientation is small or may even disappear. The following theoretical analysis only considers the period from the beginning of intra-row leaf overlapping until full canopy closure.

For the sake of simplicity, the volume of space occupied by a crop in a row can be estimated by assuming it to be a regular three-dimensional figure, which is circular, elliptical, rectangular or triangular in cross-section (Boote and Loomis, 1991). Considering the shape of sunflower plants, a trapezoid is suitable for simulating the cross-section (Fig. 1). Here, the upper width is designated as 2w, the lower width as 2W and the height of the plant as H.

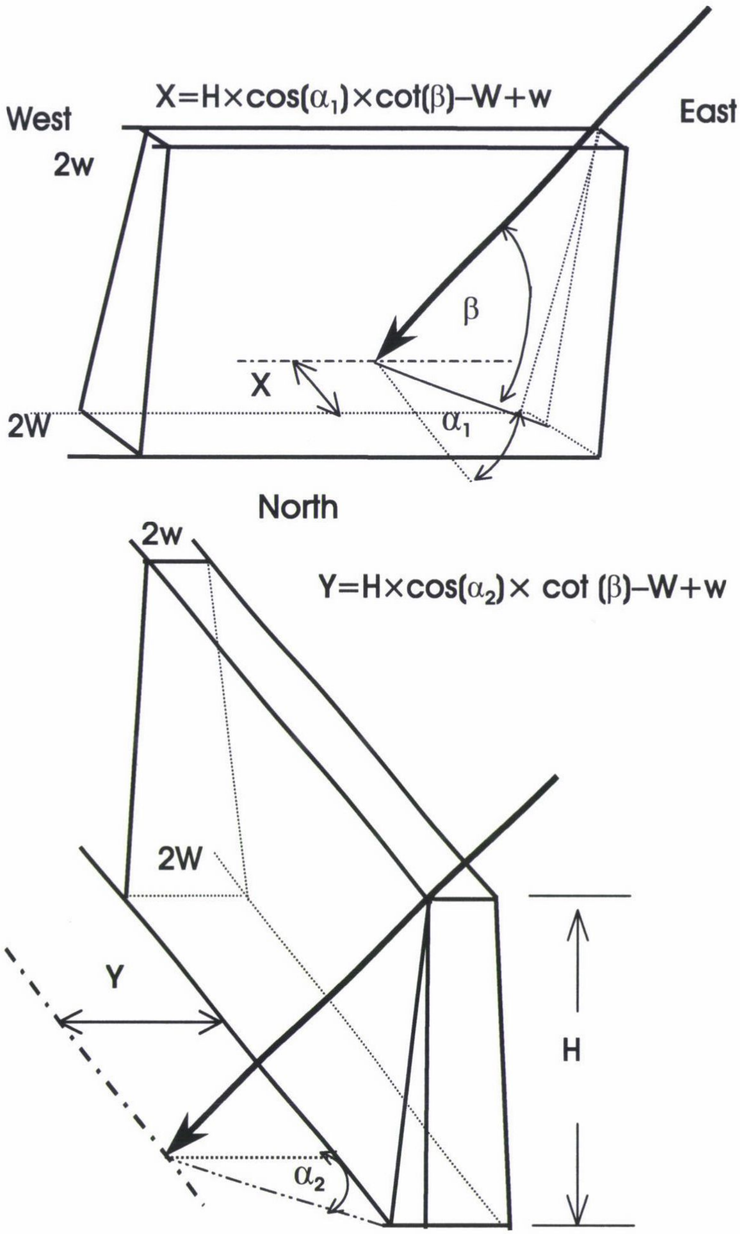

Fig. 1. Shaded ground area as a function of the altitude and azimuth angles of the sun and the canopy size for east-west (A) and north-south rows (B). β=altitude angle, r=azimuth angle, $\alpha_1=180-r$, $\alpha_2=90-r$; 2W and 2w are the width of the upper and lower sections; H is the plant height, X and Y are the maximum length of the shade

How the length of the shadow is linked to row orientation, latitude, day of the year, time of day, plant height and thickness (two functions) is illustrated in Fig. 1. X and Y represent the distances of the edges of the shadows from the east-west and north-south oriented rows, respectively. If the shadow length (X or Y) is greater than the row spacing, the soil surface is completely shaded, which indicates a fully developed canopy.

With a hypothetical crop covering 50% of the ground, Idso and Baker (1967, Fig. 2) found an effect of row orientation on light interception throughout the day. For the case of north-south row orientation, the soil was fully shaded at sunrise. Later (about 7:00 h solar time), the rays of the sun fell upon the soil surface in a thin band. Then, the sunlit band became broader reaching a maximum at noon. For the case of east-west row orientation, the situation was different. At sunrise, the rays of the sun fell upon the soil between the crop rows. This sunlit band became rapidly larger until 7:30 h, then became somewhat narrower until noon. Collectively, row orientation can play a role in light distribution within the canopy.

3.2. Experimental data

Row orientation is an often debated point in sunflower crops, and data from field experiments are still inconclusive. Robinson (1975) reported that sunflower grown in Minnesota in north-south and east-west rows did not differ in achene yield, achene oil concentration, 1000-achene weight or test weight, or in the distribution of achene size. Likewise, Myers and Minor (1993) found that there was no yield difference between the east-west and north-south row orientations. Schmidt (1995) reported that the row orientation had little effect on

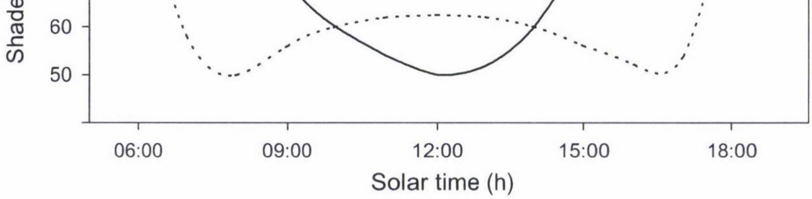

Fig. 2. Variation in shaded ground area for the east-west and north-south row orientations. The canopy covers 50% of the ground (latitude 45°N, date July 22; after Idso and Baker, 1967, adapted)

yield in the USA; however, prevailing winds might cause lodging if the rows were planted across the wind. Recently, Diepenbrock et al. (2001) found that the east-west row orientation produced higher yields than the north-south row orientation. The effect of row orientation was significant at P=0.05 in 1996 and 1998. Lodging may affect the influence of row orientation, but the differences in achene yields were not attributable to variation in lodging. In contrast to sunflower, higher light interception in north-south rows than in east-west rows was detected for cotton (Baker and Meyer, 1966), maize, wheat (Donald, 1963) and soybean (Philbrook and Oplinger, 1989). In part, conflicting results may be explained by the fact that the studies were conducted at different latitudes.

4. Conclusions

The following conclusions may be drawn. First, experiments dealing with the effects of plant density and row spacing on yield traits and other agronomic parameters gave inconsistent results. Thus far, the reasons for this have not been well elucidated. To fill this gap in knowledge, more field experiments combined with modelling approaches are required. Second, sound information on the response of sunflower to row orientation is meagre. Research on the effects of row orientation and the interactions between row orientation, row spacing and plant density may help to maximize yield.

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Book review

RODNEY W. BOVEY (2001): Woody Plants and Woody Plant Management – Ecology, Safety, and Environmental Impact. Marcel Dekker, Inc., New York – Basel
564 pages, hardback - ISBN: 0-8247-0438-X
website: http://www.dekker.com

The management of woody plants has long been a challenge for mankind. Increasing the production of fruits for food, increasing timber production for various uses, and the management of woody vegetation in order to decrease soil erosion were and still are very important issues. This book by Rodney W. Bovey – who is an Adjunct Professor in the Department of Rangeland Ecology and Management, Texas A & M University, College Station, and the author or co-author of more than 300 articles, book chapters and books – has a different approach. It is about managing the woody plants which are undesirable for wildlife, livestock, or agriculture and timber production. It is very easy to recognise that this aspect is of no less importance. Particularly if we consider that woody plants are represented even among the aggressively invading "weed" species and that billions and billions of all currencies are spent year by year to control them. The main aim of this book is to bring together the extensive but scattered information on woody plant management over the last 50 years.

As an introduction, in the 1st chapter the author gives an overview of the significance and botanical nature of woody plants with emphasis on the characteristics particularly important from the point of view of different management approaches. In the 2nd chapter there is a historical overview of woody plant management. It seems quite evident that in the last 50 years chemical control has been the dominant approach, followed by fire, so chemical control technologies are given a prominent place in the book. The following chapters discuss the development of herbicides, application technologies, herbicide toxicology, ecological impacts, etc. in detail. Separate chapters are devoted to the economic evaluation of woody plant management and the non-chemical methods of woody plant control. This last chapter discusses mechanical and biological (insects and pathogens) control technologies and fire as control agents.

The book describes the properties and impacts of herbicides on both the target woody plants and the environment. It analyses the responses of close to 400 woody plant species to the different herbicides available commercially.

Although the book is almost exclusively about woody plant management in North America – the examples given refer to North American species and situations – there is much to be learnt from it, and it is recommended to all those who have to deal with the management of woody plants either in forests or in agricultural ecosystems.

G. CSÓKA

Erratum
The tables on pages 225 and 255 of Issue No. 3 of Volume 49, 2001 were regrettably substituted for each other. The editors apologise for any inconvenience.

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VOLUME 49, 2001

AKADÉMIAI KIADÓ, BUDAPEST
2001

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2. Every original standard paper should be divided into the following **sections:** Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References. Manuscripts should be headed with the **title** of the paper, initial(s) of first name(s) and surname(s) of author(s), and the institute where the research was carried out. A **running title** not to exceed 50 letter spaces should be included on a separate sheet.

3. **Abstracts** are required for all the manuscripts. They should be limited to max. 200 words. Up to 8 **key words** should be added at the end of the abstract.

4. Genus and species **names, gene symbols** and **Latin words** are printed in *italics.* A single straight line should be drawn under such names if no italic script is available.

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Kiss, G., Papp, I., Bakondi-Zámori, E., Gartner-Bánfalvi, Á. (1977): A szója fungicides magcsávázásának és rhizóbium oltásának együttes tanulmányozása. (Joint study of fungicide dressing and rhizobium inoculation in soybean.) *Növénytermelés,* **26,** 147–153.

Ouyang, J. (1986): Induction of pollen plants in *Triticum aestivum.* In: Hu, M., Yang, M. (eds), *Haploids of higher plants* ***in vitro***. Academic Press, Beijing, 26–41.

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